Modelling the Population Dynamics of *Ascaris suum* in Pigs

by

Shana Coates

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Danish Centre for Experimental Parasitology,
The Royal Veterinary and Agricultural University, Copenhagen,
Denmark

and

Department of Biological Science,
The University of Warwick, UK

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In memory of Stafford Coates

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DECLARATION

This thesis is the result of original research conducted by myself unless stated in the text or acknowledgements. The research was carried out under the supervision of Dr. Graham Medley at the University of Warwick and Dr. Allan Roepstorff at the Royal Veterinary and Agricultural University in Denmark. All sources of information have been specifically acknowledged.

No part of this thesis has been submitted for a degree at any other university.

Work from Chapter 2 has previously been published in:

Boes, J., Coates, S., Medley, G.F., Varady, M., Eriksen, L., Roepstorff, R. & Nansen, P. (1999). Exposure of sows to *Ascaris suum* influences worm burden distributions in experimentally infected suckling piglets. *Parasitology* **119**, 509-520.

ABBREVIATIONS

DCEP	Danish Centre for Experimental Parasitology
EPG	Eggs per gram of faeces, sensitivity 20 EPG
IMM	Immature worms
LTS	Liver texture score: 1 normal
	2 moderate
	3 severe
p.i.	Post inoculation
p.i.i.	Post initial inoculation
SPF	Specific pathogen free
Un-ID	Unidentified gender for adult *A. suum*
WS diffuse	Diffuse liver white spots
WS lyph.	Lymphnodal liver white spots

SUMMARY

The objective of this thesis was to examine factors that influence the population dynamics of *A. suum* infections in pigs. In particular, the role of exposure in determining the pattern of aggregation of the parasite was addressed.

In Chapter 2 data from a series of experiments investigating the effect of maternal exposure on the infection of offspring with *A. suum* were used to examine changes in the worm burden distribution, and the relationship between the aggregation parameter, k, and the mean intensity of infection. Analysis by maximum likelihood demonstrated that the colostrum of previously infected sows caused the distribution of worms among their piglets to become less aggregated, the degree of which determined by the length of exposure of the sow. A linear relationship between the aggregation parameter and the mean intensity of infection was also found.

In Chapter 3, further experimental data from DCEP was compiled and used to examine the effect of inoculation protocol on the worm burden distribution. It was shown that trickle inoculations mimicked natural infections well, whilst single inoculations had a lower prevalence and mean intensity and a higher degree of aggregation.

Chapter 4 describes an experiment which was designed to investigate the effect of exposure on aggregation, using a trickle inoculation protocol. The design incorporated additional investigations into the effect of inoculation dose level and duration of infection. Host predisposition, the effect of experience on parasite fecundity, and the development of a pre-hepatic barrier to infection were also examined. The new experimental results contradicted those found in a natural infection / reinfection experiment, and opposed the hypothesis that experience of infection causes a reduction in the aggregation of the worm burden distribution. However, a significant predisposition was found, experience of infection was shown to reduce the parasite fecundity, and larvae were shown to continue to migrate during a trickle inoculation. The experimental results also demonstrated that coproprevalence changed through time as a function of dose level.

The relationship between coproprevalence and dose was used to develop a dynamic model (chapter 5). The model was used to demonstrate that the force of infection in the natural reinfection experiment was likely to have been considerably greater than those used in the trickle inoculation experiment. Although initially the immune response is greater in animals experiencing a high force of infection, through time a high force of infection leads to greater unresponsiveness.

CHAPTER 1: INTRODUCTION

1.1 *ASCARIS SUUM*

Ascaris suum Goeze1782, commonly known as the large roundworm, is a nematode found in the small intestine of the pig. It is one of the most prevalent pig nematodes (Murrell, 1986), and also one of the largest. Adult female worms can measure up to 49 cm in length and the males can measure up to 31 cm in length (Roberts & Janovy, 1996). The males are distinguished from the females by their smaller size and curved posterior end, see fig 1.1. As a result of its large size *A. suum* is easy to detect and identify, which together with its wide prevalence has led to it being the subject of much research.

Life Cycle

The life cycle of *A. suum*, illustrated in figure 1.2, is direct. The basic migration of larvae from the intestine via the liver and lungs back to the intestine has previously been described by Roberts (1934), Kelly *et al.* (1957), Douvres *et al.* (1969) and Douvres & Tromba (1971), although more recent work has clarified the preferred site of intestinal penetration (Murrell *et al.* 1997) and the relative spatial and temporal distribution of larvae during the period of migration (Roepstorff *et al.* 1997). The accepted life cycle of *A. suum* is as follows. Ingested infective eggs hatch in the small intestine, the larvae then penetrate the wall of the cecum and upper intestine and within six hours the migrating larvae appear in the liver. Approximately seven days after the initial ingestion, the majority of larvae have migrated from the liver to the lungs.

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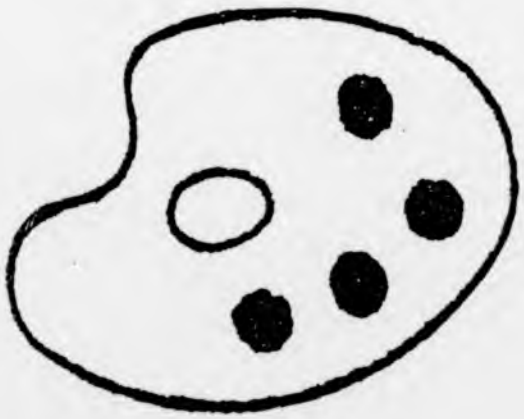

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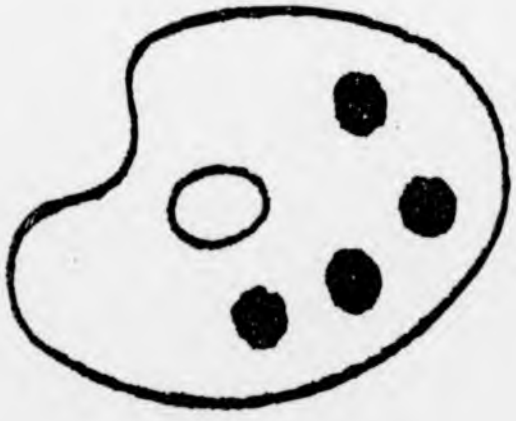


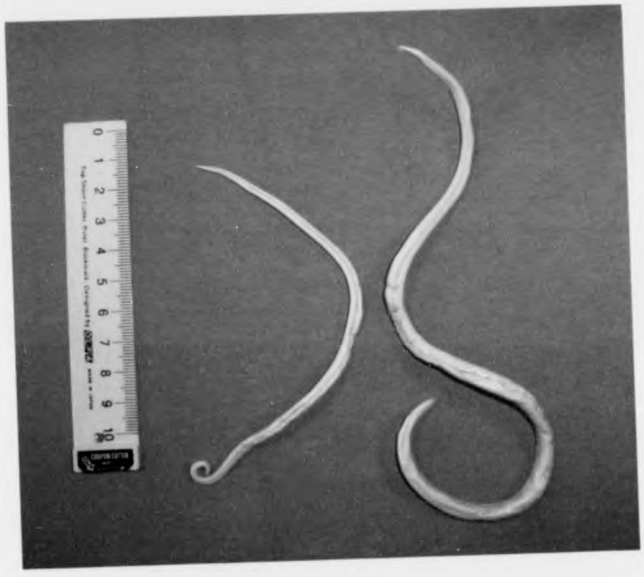


Figure 1.1 Male (left) and female *Ascaris suum*. The posterior end is facing downwards. The male is distinguished from the female by its curved posterior end and smaller size. A ten *cm* ruler is shown for perspective.

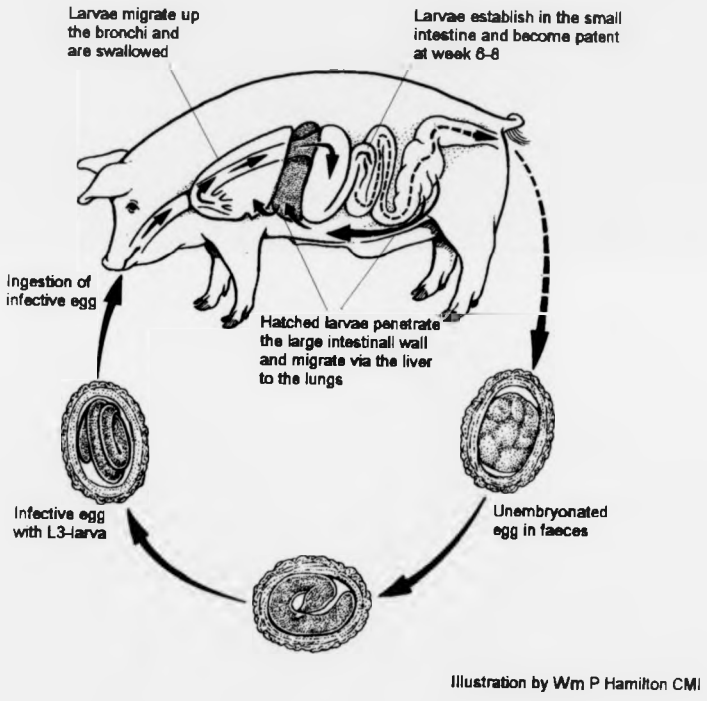


Figure 1.2 The life cycle of *Ascaris suum* (Roepstorff & Nansen, 1998)

Subsequently they migrate up the trachea to be swallowed again, from where they return to the small intestine approximately 10 days post-initial-ingestion. Between days 14 and 21 post-initial-ingestion, the majority of worms are expelled from the small intestine (self-cure), resulting in small over-dispersed populations by day 28. The remaining worms mature in the small intestine, and become patent approximately 6 - 8 weeks after infection (initial ingestion). A total of four moults occur during the life-cycle. Recent evidence suggests that the first two moults occur in the egg and the final two moults occur in the digestive tract post-migration (Fagerholm *et al.* 2000).

Egg Production

The fecundity of *A. suum* is immense. It has been estimated that each female is capable of producing up to two million eggs per day (Olsen *et al.*, 1958). Once in the environment the eggs take approximately 5 weeks to become infective under favourable conditions (Larsen & Roepstorff, 1999). Development ceases when the maximum temperature falls below 15 °C (Seamster, 1950), though the eggs are able to survive (Stevenson, 1979). They may remain viable in the environment for up to 10 years (Roepstorff & Nansen, 1994), due to a thick shell consisting of four layers (Wharton, 1980) which makes the eggs highly resistant.

Diagnosis

The presence of eggs in the faeces is a commonly used method to diagnose infection with *A. suum* (eg. Roepstorff & Jorsal, 1989, 1990). This method is not 100% sensitive, as both the male and female should be present for egg excretion to occur (Jungersen *et al.* 1997). However the most heavily infected hosts will almost certainly be detected and it

is these that are at the highest risk of morbidity and most likely to contribute to the further transmission of infection. The specificity of faecal eggs counts is affected by low numbers of eggs that may be present in the absence of a patent infection and are most likely due to coprophagia or geophagia (Boes *et al.* 1997). Roepstorff (1997) regarded all egg counts of less than 200 eggs per gram of faeces (EPG) as false positive. Typically a single mated female may produce 400 - 800 EPG in growing pigs (Roepstorff & Nansen, 1998).

Pathogenesis & Economic Importance

Morbidity due to *A. suum* infection is usually subclinical, although pneumonia may occur during the migration of larvae through the lungs, and intestinal obstruction may occur in animals with exceptionally high worm burdens (Urquhart *et al.* 1996). The migration of larvae through the liver can produce an inflammatory reaction which results in "white spot" lesions (Ronéus, 1966). The white spots fade approximately 4 to 6 weeks after the larvae have left the liver (Eriksen *et al.* 1980), and are used as an indication of recent infection. Severe liver white spots can result in the condemnation of the liver at slaughter (Roepstorff & Nansen, 1994), contributing to production losses. Another important production loss caused by *A. suum* infection is reduced feed to gain ratios (Stewart & Hale, 1988). It was estimated that in 1994 the economic loss in the United States due to *A. suum* infection was $174.3 million (Stewart, 1996).

Control

Control of *A. suum* and other intestinal parasites is an important prerequisite for profitable swine production (Roepstorff, 1991). Anthelmintic treatment has a direct

effect on the survival of parasites within the host, however when used alone it has a transitory influence on the excretion of eggs, as hosts rapidly reacquire infection (Nilsson, 1982). Effective management and hygiene act to reduce the development and survival of the eggs in the environment, but due to the resilient nature of the eggs it is extremely difficult to eradicate them totally. It has been suggested that the optimum approach would be to integrate anthelmintic treatment with management and hygiene practices to avoid the development of anthelmintic resistance (Roepstorff & Nansen, 1994). Under conditions of intensive management it may be possible to replace the routine use of anthelmintics with coprological surveillance combined with anthelmintic treatment when necessary (Roepstorff, 1997).

1.2 *ASCARIS LUMBRICOIDES*

The equivalent human parasite *A. lumbricoides* Linnaeus 1758 is equally ubiquitous. Much debate has taken place as to whether *A. suum* and *A. lumbricoides* are in fact separate species, with investigations centred on comparisons of morphology, chromosomes, physiology, biochemistry, antigenicity and courses of infection in man and pigs, see Crompton (1989a, 2001). Whether or not the parasite infecting pigs is epidemiologically and genetically distinct from that infecting humans is important for the design of control programmes and for the management of drug resistance, as in many countries both hosts live in close proximity and both may be infected with *Ascaris* (Anderson *et al.* 1993a, Peng *et al.* 1996). A strong association has been shown between households owning pigs and containing heavily infected individuals (Anderson *et al.*

1993b). Experimental cross-infections have shown that it is possible for human-derived worms to infect pigs (Galvin 1968) and molecular analysis has incriminated pigs as the source of 9 cases of *Ascaris* infection in humans in North America (Anderson, 1995). However, molecular evidence from sympatric populations of *Ascaris* infecting humans and pigs suggests that the gene flow between the two populations is limited and in effect, there are two separate host-specific transmission cycles (Anderson *et al.* 1993a). The question will only be resolved when an experiment proves that the two species can interbreed and produce fertile offspring.

Prevalence

Chan *et al.* (1994) estimated that *A. lumbricoides* infected 1,472 million people worldwide. The robustness of this estimate is supported by three independent estimates of *A. lumbricoides* infections in China (Crompton, 1999). Chan *et al.* (1994) estimated that 568 million humans were infected with *A. lumbricoides* in China, Xu *et al.* (1995) and Peng *et al.* (1998a) estimated this to be 531 and 532 million people respectively. The majority of infections are located in developing countries (Crompton, 1989b). In contrast, approximately 1,000 cases are reported annually in the United Kingdom (Owen, 1986). It is generally not known whether these cases derive from contact with pigs or travel abroad.

Morbidity and Mortality

Diagnosis of ascariasis is almost exclusively made by coprological examination, although the expulsion of worms following anthelmintic treatment is also used (Crompton 1989b). de Silva *et al.* (1997) classified disease levels for *A. lumbricoides*. Light infection may

cause reversible growth limitation and reduced physical fitness, moderate infection may cause permanent growth retardation, whilst severe infection may result in clinically overt illness (nausea, anorexia, diarrhoea), acute complications (intestinal obstruction, appendicitis, peritonitis) and mortality. It has been estimated that ascariasis results in a global disability-adjusted life year (DALY) loss of 10.5 million, contributing to a total global DALY loss of 39.0 million as a result of infection with intestinal helminths. This compares to an estimated global DALY loss of 35.7 million for malaria and 34.1 million for measles (Chan, 1997).

1.3 BACKGROUND TO RESEARCH

Ascaris suum infections in the pig are characteristically aggregated in their hosts (Boes, 1999), which implies that the majority of the parasites are harboured by the minority of the hosts (Anderson, 1987). This has been observed in infections that derive naturally from contaminated pasture (Roepstorff & Murrell, 1997), from single experimental inoculations (Roepstorff *et al.*, 1997) and from trickle inoculations (Eriksen *et al.*, 1992b). A highly aggregated distribution is also observed in the comparative human infection, *A. lumbricoides* (Hall *et al.* 1992; Chai *et al.*, 1985) and appears to be a common feature of most intestinal helminth infections (Shaw *et al.* 1998; Shaw & Dobson, 1995; Anderson, 1985; Anderson & May, 1985). Studies on factors that influence the degree of aggregation in *A. lumbricoides* infections have shown that the aggregation remains high within groups stratified by host age (Thein-Hlaing *et al.* 1984; Bundy *et al.* 1987) and host gender (Haswell-Elkins *et al.* 1989).

The frequency distribution of numbers of worms per host is well described by the negative binomial probability distribution (Bliss & Fisher, 1953), which has proved to be a good empirical model (Anderson & Medley, 1985). This distribution is defined by the mean worm burden, M, and an aggregation parameter, k, which is inversely related to the degree of aggregation. As k becomes smaller the distribution becomes more aggregated. Typical values of k are less than unity for both *A. suum* and *A. lumbricoides* infections (Roepstorff *et al.* 1997, Boes *et al.* 1998, Guyatt *et al.* 1990).

The mechanism generating aggregation of the worm burden distribution has been attributed to various processes, such as variability between hosts in resistance to infection, development of immunity, behaviour and age. The non-random spatial distribution of infective stages and non-random host encounters with infective stages have also been proposed. For a review see Anderson & Gordon (1982). Both the rate and duration of infection are likely to influence the degree of aggregation of the number of worms per host, resulting in a frequency distribution that is both -age and intensity-dependent (Keymer & Pagal, 1990). Probability theory has been used to conclude that over-dispersion generated by intrinsic host heterogeneity has components that remain constant through time (between an initial infection period and a reinfection period) and components that are more transitory (McCallum, 1990). The mechanisms by which immunity operates and the site within the body at which it is manifest both remain unknown, however it has been shown that natural immunity to *Ascaris* in humans is associated with IgE antibody to one the parasite's major allergens (McSharry *et al.* 1999). Another human study has shown that between 30% and 50% of the variation in the worm burden could be accounted for by genetic components, providing further

evidence for the importance of host genetic factors in the determination of *Ascaris* worm burdens (Williams-Blangero *et al.* 1999).

It has been predicted that a moderate degree of aggregation is required to create stability in the host-parasite system (Anderson, 1978). A model by Adler & Kretzschmar (1992) further suggested that the stability resulted from the dependence of dispersion on the mean, however computer simulations did not support this finding. It was suggested that a more complex model that allowed the mean and negative binomial parameter, k, to vary independently may be more appropriate (Adler & Kretzschmar, 1992).

1.4 OBJECTIVES OF THE STUDY

An aim of this study was to undertake a wide range of techniques and approaches whilst investigating the population dynamics of *Ascaris suum*. Specifically, this included the following:

- to collect and interpret data from previous experiments conducted by others at the Danish Centre for Experimental Parasitology (DCEP)
- to examine factors, such as maternal experience of infection and inoculation protocol, that may affect the degree of aggregation in the distribution of *A. suum* among pigs

- to design and execute an experiment to test the hypotheses generated from the initial analysis, regarding factors that influence the dynamic nature of parasite aggregation, and conduct post-mortem examination
- to incorporate additional studies into the experimental design to maximise the potential for new results
- to perform appropriate statistical analysis of the data sets and interpret the results
- to generate simulated data sets
- to develop a mathematical model to explore the population dynamics of *Ascaris suum*, and to fit data to the model

CHAPTER 2: THE RELATIONSHIP BETWEEN THE DEGREE OF AGGREGATION AND THE MEAN INTENSITY OF *A. SUUM* INFECTIONS IN PIGLETS, AND THE EFFECT OF MATERNAL EXPOSURE

2.1 INTRODUCTION

Ascaris infections are typically aggregated among their hosts (eg. Eriksen *et al.* 1992b, Holland *et al.* 1989) and like other macro-parasites are well described empirically by the negative binomial distribution (Wilson *et al.* 1996). This distribution is defined by the mean intensity of infection and a parameter, k, which represents the inverse aggregation: as k becomes smaller, the distribution becomes more aggregated (Anderson & May, 1982).

In the present study, observed *A. suum* worm burdens were fitted to the negative binomial distribution using the maximum likelihood method (Hilborn & Mangel, 1997b; Williams & Dye, 1994). The mean intensity of infection was estimated directly from the data, whilst the value of k was derived as the value that gave the best fit in terms of the observed worm burdens. This was done, firstly to examine whether the negative binomial aggregation parameter, k, changed as a function of the mean worm burden, and secondly, to assess how different experimental conditions affected the aggregation of the worm burden distribution. The data came from a series of experiments designed to investigate

if the exposure of sows to *A. suum* influenced subsequent experimental *A. suum* infections in their piglets compared with piglets from helminth-free control sows (Boes *et al.* 1999).

The relationship between k and the mean worm burden has previously been investigated for endemic infections. It has been shown that for *A. lumbricoides* infections in human communities, k is best represented as a linear function of the mean intensity of infection (Guyatt *et al.* 1990). Other parasitic systems, when fitted to the negative binomial distribution, have been best described where k is an exponential function of the mean (Medley *et al.* 1993). The aim of the present study was to examine the factors that influence the degree of aggregation newly acquired (rather than endemic) infections, attained under controlled experimental conditions.

2.2 METHOD

For the negative binomial distribution, the probability, p, of an individual harbouring w worms, given a mean worm burden, m, is:

$$p(w,m,k) = \frac{\Gamma(k+w)}{\Gamma(k)w!}\left(\frac{m}{k+m}\right)^{w}\left(1+\frac{m}{k}\right)^{-k} \qquad \textbf{(2.1)}$$

where Γ refers to the Gamma function.

The likelihood of an individual harbouring w_i worms, where w_i is the observed worm burden for individual i, was calculated. The log-likelihood was summed across all individuals, and k was estimated for the distribution by maximising the total log-likelihood:

$$L\{w|k,m\} = -\log\left(\frac{\Gamma(k+w)}{\Gamma(k)w!}\left(\frac{m}{k+m}\right)^{w}\left(1+\frac{m}{k}\right)^{-k}\{\text{terms not in } p\}\right) \quad \textbf{(2.2)}$$

The relationship between k and m_j, where m_j is the mean worm burden for group j, was investigated by defining k as a function of m, according to the following equations:

$$\text{Constant: } k(m) = a_0 \quad \textbf{(2.3)}$$

$$\text{Linear: } k(m) = a_0 + a_1 m \quad \textbf{(2.4)}$$

$$\text{Exponential: } k(m) = a_0\left(1 - \exp\left(-\left(a_1 m\right)^{a_2}\right)\right) \quad \textbf{(2.5)}$$

where a_0, a_1 and a_2 are parameters.

The data set used in this study comprises of the worm burdens from 178 experimentally infected piglets, grouped by sow. The piglets were infected with either two or three doses of 50 eggs within two weeks of birth and necropsied at 10 weeks of age (for

experimental details see Boes *et al.* 1999). The data set can be divided into subsets according to different criteria, such as the experiment from which the data came, whether the sow had been exposed to *A. suum* or not, and the length of exposure to *A. suum* that the sow experienced. Details of the subsets are given in table 2.1. In addition to examining k as a function of m, the changes in k across the different subsets were also investigated, to obtain the optimal description of the data. The significance between the different functions of k and the different descriptions of the data were tested using the likelihood ratio test, when the models were nested[1]. When the models were not nested, e.g. when comparing the linear function with the exponential function, the Akaike information criterion (AIC) was used.

The likelihood ratio test is used to test between competing models of increasing complexity. It resulted from statistical theory by Kendall & Stewart (1979) which demonstrated that the difference in likelihood (or the ratio of log-likelihoods) between two competing models had a chi-squared distribution, with degrees of freedom equal to the difference in the number of parameters. The AIC is given by the sum of likelihood plus twice the number of parameters. The best model is selected by the lowest AIC (Akaike, 1992).

A family of models are referred to as nested if the simper models are special cases of the more complex models. For example, models are nested if, by setting one or more parameters to zero, the more complex model collapses into the simple model. Non-nested models are structurally different from each other (Hilborn and Mangel, 1997c).

Table 2.1 The origin of the experimentally infected piglets used in this analysis. For further experimental details, see Boes *et al.* (1999).

Experiment	Sow, j	Exposure of sow to *A. suum*, in months	No. of piglets born to sow	Cross-suckling information: no. of piglets donated (to sow)	Cross-suckling information: no. of piglets received (from sow)
1	L1	0	8		
	L2	0	4		
	L3	0	9		
	L4	0	10		
	L5	6	9		
	L6	6	11		
	L7	6	11		
	L8	6	7		
	L9	6	9		
2	S1	0	6		
	S2	0	7		
	S3	0	9		
	S4	3	11		
	S5	3	10		
	S6	3	11		
	S7	3	12		
3	A	0	7	4 (to D)	9 (from D)
	B	0	7	2 (to C)	4 (from C)
	C	3	7	4 (to B)	2 (from B)
	D	3	13	9 (to A)	4 (from A)

For the negative binomial distribution, the prevalence of infection can be expressed in terms of the mean intensity and the aggregation parameter, k:

$$P = 1 - \left(1 + \frac{m}{k}\right)^{-k} \qquad \textbf{(2.6)}$$

This expression was used to illustrate the relationship between the prevalence and the mean, and the effect of aggregation, for the sow groups used in this analysis.

For each function examined, the estimated parameters (a_0, a_1, a_2) were compared across all possible subsets, where the subsets comprised of the experiment and/or the length of exposure the sow had to *A. suum*. In addition, where more than one parameter was estimated for a function, the possibility that not all parameters varied across the different experiment criteria was also investigated. For example, when examining the effect of exposure on k as a linear function of the mean, the following scenarios were considered:

(i) both parameters change with exposure;
(ii) the slope changes with exposure whilst the intercept remains constant;
(iii) the intercept changes with exposure whilst the slope remains constant.

To accommodate data from a cross-suckling experiment, the analysis was performed twice. Once when the piglets were grouped according to the sow that they suckled and once when they were grouped by their biological mother.

2.3 RESULTS

This work has been published by Boes *et al.* (1999). Discrepancies between the published work and the following results are due to the accidental exclusion of one piglet from the analysis presented in the published work. The piglet belonged to short exposure sow 4, had a worm burden of three, and contributed a log-likelihood of approximately 3 (depending on the hypothesis being tested) to the total log-likelihood. In the paper, the number of piglets belonging to this sow is given as 9, the actual number is 10. There was no noticeable difference to the mean worm burden as $31/9 \approx 34/10$.

By allowing the parameters of a function to vary separately across exposures, the linear function became optimal for both sets of data as the number of parameters in the likelihood ratio test was reduced.

In the paper one piglet belonging to sow C in the suckle analysis was given a nominal worm burden of one. In the present analysis, the likelihood was estimated based on the probability of piglets having no worms when the mean worm burden was zero.

2.3.1 Piglets grouped according to the sow that they suckled

When the piglets were grouped by their suckle mother, one group had a mean worm burden of zero. It is not possible to compute the likelihood of a piglet having a worm burden of zero given a mean of zero, however, as the mean tends to zero, the likelihood

of an individual having no worms tends to 1. Piglets in this group were therefore given a probability of one to enable us to compute the total log-likelihood for the whole data set.

The best description of the data for each function is given in table 2.2. The optimal model for the data, is given by k as a linear function of the mean intensity of infection for each sow group, where the intercept is dependent on the length of exposure that the sow had to *A. suum*, and the slope is common across all exposures. The control group represents a coalescence of the piglets that suckled *Ascaris*-naive sows from all three experiments.

Figure 2.1 gives the 95% confidence region for the best fit parameters of k as a linear function of the mean intensity of infection. This was computed by searching over all values of a_0 and a_1 that provide negative log-likelihoods with a value of 3.0 (half the critical value of the χ^2 distribution with two degrees of freedom) greater than the minimum. Figure 2.2 (a - c) shows the relationship between k and the mean intensity for all three functions, and the best description of the data for each function.

Table 2.2 Results of fitting the negative binomial distribution to the data, varying k as a function of the mean intensity of infection. The best description of the data according to different experiment criteria is given for each function. The cross-suckle data is grouped by suckle mother

Function (equation)	Negative Log-likelihood	p-value	Best description of data	a_0	a_1	a_2
Constant (2.2)	517.5456		Control Short Exposure Long Exposure	0.305 0.613 1.423		
Linear* (2.3)	512.9432	0.002	Control Short Exposure Long Exposure	0.074 0.479 1.223	0.019	
Exponential (2.4)	515.8902	ns	Control Exposed	1.157 2.587	0.017	0.772 0.372

*Optimal model

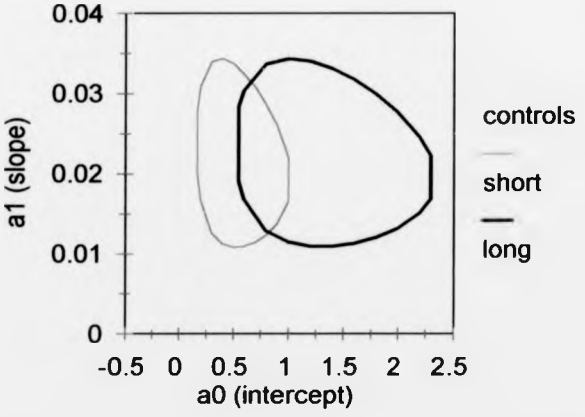
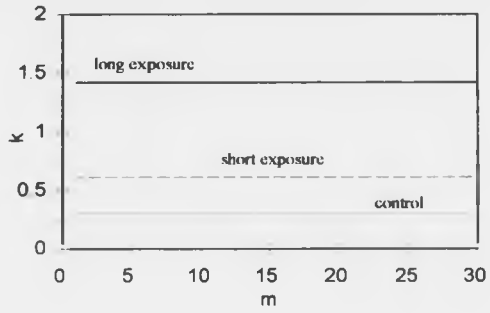
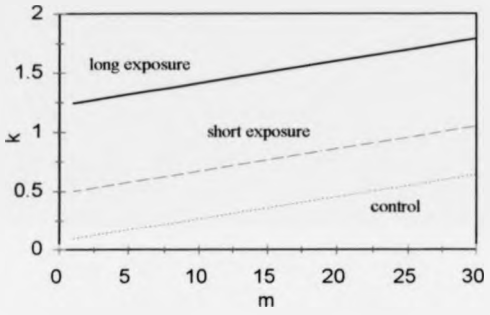


Figure 2.1 The 95% confidence region for the best fit parameters of k as a linear function of the mean intensity of infection. Grouped by suckle mother.





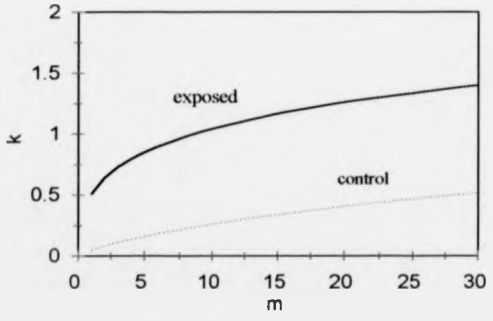


Figure 2.2 The best fit of the data, when piglets are grouped by suckle mother, for the (a) constant, (b) linear, and (c) exponential relationships between k and the mean intensity of infection

2.3.2 Piglets grouped by biological mother

The best description of the data for each function is given in table 2.3. The optimal model was again given by k as a linear function of m, with a common slope. However, when grouped by biological mother, the intercept was only dependent on whether or not the sow had been exposed to *A. suum* for a long duration.

Table 2.3 Results of fitting the negative binomial distribution to the data, varying k as a function of the mean intensity of infection. The best description of the data according to different experiment criteria is given for each function. The cross-suckle data is grouped by biological mother

Function (equation)	Negative Log-likelihood	p-value	Best description of data	a0	a1	a2
Constant (2.2)	531.2279		Control / Short Exposure Long Exposure	0.312 1.423		
Linear* (2.3)	527.8858	0.0078	Control / Short Exposure Long Exposure	0.139 1.220	0.017	
Exponential (2.4)	528.8648	ns	Control / Short Exposure Long Exposure	6.416 25.892	6.3×10^{-5}	0.399

*Optimal model

Figure 2.3 shows the 95% confidence region for the best fit parameters of k as a linear function of the mean intensity of infection. Figure 2.4 (a - c) shows the best description of the data for the three functions of k given in table 2.3.

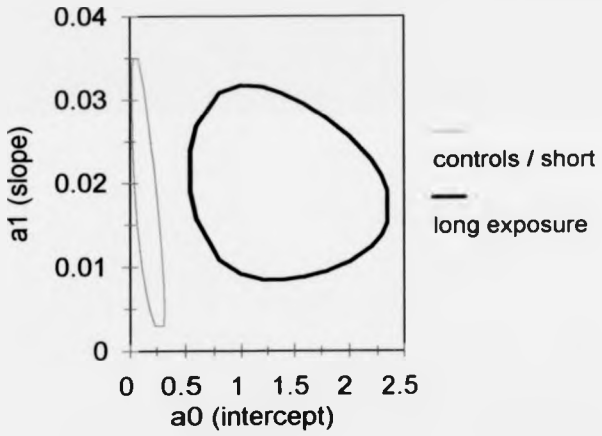


Figure 2.3 The 95% confidence region for the best fit parameters of k as a linear function of the mean intensity of infection. Grouped by biological mother

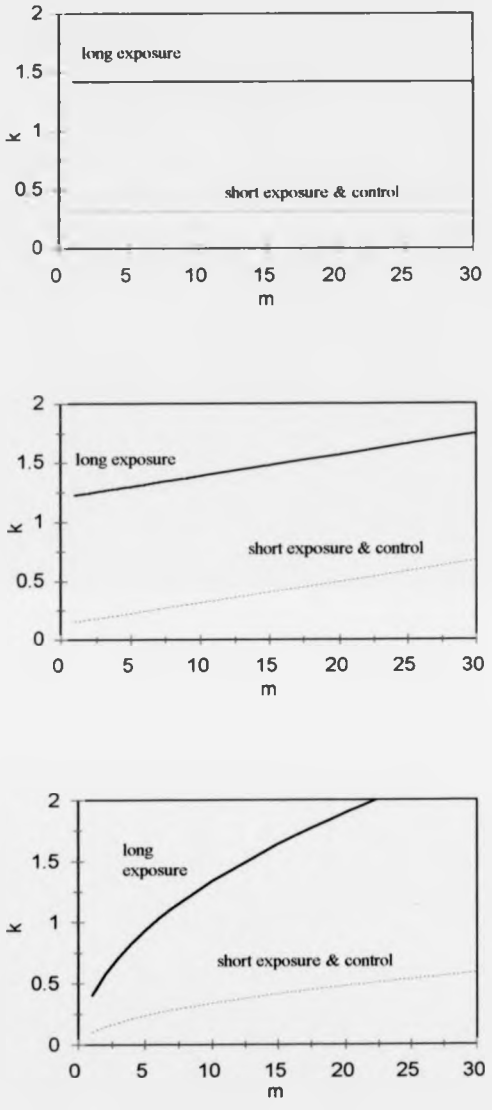


Figure 2.4 The best fit of the data, when piglets are grouped by biological mother, for the (a) constant, (b) linear and (c) exponential relationship between k and the mean intensity of infection

2.3.3 The relationship between the mean worm burden and the prevalence of infection

Figure 2.5 shows the relationship between the mean worm burden and the prevalence of infection for the suckle mother data, as predicted by the negative binomial distribution where k is a linear function of the mean, and the intercept is determined by the duration of the sows' exposure to *A. suum*. Each data point represents a litter of piglets belonging to one sow.

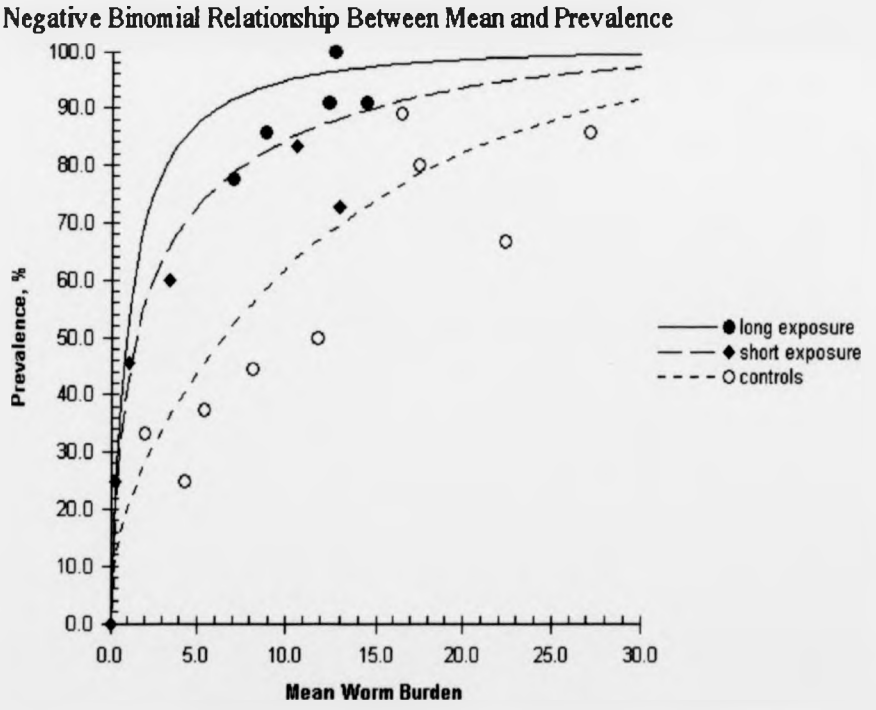


Figure 2.5 The relationship between the mean worm burden and the prevalence of infection as given by the negative binomial distribution using the optimal description of k for the suckle mother data.

2.4 SUMMARY

2.4.1 The aggregation parameter, k, as a function of the mean intensity of infection

Regardless of whether the piglets were grouped with their natural mother or their suckle mother, the relationship between the negative binomial aggregation parameter and the mean intensity of infection was best described by a linear function. The slope was positive and common across all subsets of the data, thus as the mean intensity of infection increased the distribution became less aggregated.

2.4.2 The effect of sow exposure to A. suum on the distribution of worms among piglets

When the piglets were grouped by their suckle mother, the intercept was dependent on the length of time the sow had been exposed to *A. suum*. The longer the exposure of the sow, the less aggregated the distribution among offspring. When the piglets were grouped by their biological mother, the short exposure group and the control group converged in their aggregation, resulting in a best fit given by one intercept representing both control and short exposure animals and a second intercept representing the long exposure animals. Again the long exposure animals had a less aggregated distribution.

2.4.3 Comparison of suckle mother and biological mother results

The exposed sows in the cross-suckling experiments were exposed for a short duration. The convergence of the intercept of the short exposure and control groups when the piglets are grouped by their biological mother compared to their suckle mother suggests that the factor that causes the reduction in aggregation is obtained from the suckle mother, via colostrum antibodies.

CHAPTER 3: THE EFFECT OF EXPOSURE ON THE DISTRIBUTION OF *ASCARIS SUUM* IN PIGS

3.1 INTRODUCTION

In the last chapter, it was shown that exposure to *A. suum* in sows can cause a reduction in the aggregation of worms distributed among their offspring. An experiment investigating the reinfection of naturally exposed pigs with *A. suum* following anthelmintic treatment (Boes *et al.* 1998) also revealed a reduction in aggregation with experience of infection. However, this reduction may be attributed to a number of factors, such as changes in the number of infective eggs on the pasture. To assess whether experience of infection was the cause, the results need to be replicated under more controlled conditions. In this study we examine inoculation protocol and the sample size required to reproduce statistically significant results, if a true effect has occurred.

In the first part of this study, the effect of different inoculation protocols on the distribution of *A. suum* worm burdens in pigs was investigated, by summarising and analysing data collected at DCEP. Data from eleven experiments were used to compare *A. suum* worm burdens from natural, experimental trickle and experimental single dose infections. The distributions were examined for the effect of exposure on the mean, prevalence and degree of aggregation.

For logistical, practical and funding reasons, the number of pigs used in an experiment is restricted. In the second part of this study, the Monte Carlo technique was used to simulate data of different sample sizes, given the distributions found in the reinfection experiment. The simulated data sets were used to estimate the probability of obtaining statistically significant results, and thus predict the sample size required to replicate the results, based on defined assumptions.

PART I: THE EFFECT OF INOCULATION PROTOCOL ON THE DISTRIBUTION OF *ASCARIS SUUM* IN PIGS

3.2.1 METHOD

Details of the experiments used in this study are given in table 3.1. The experiments were grouped according to the inoculation protocol used. The mean and prevalence of infection were calculated for each group. Prevalence of infection was compared across different methods of exposure, using Pearson's χ^2 test and Fisher's exact test. The intensity of infection was compared across the different methods of exposure using the Kolmogorov-Smirnov test. The negative binomial aggregation parameter, k, was calculated using the maximum likelihood technique. All distributions were tested for agreement with the negative binomial model using the χ^2 test. The maximum likelihood ratio test was used to distinguish between three proposed models of aggregation:

(i) The aggregation is the same across all groups;

(ii) The aggregation is the same for trickle inoculation and natural exposure, but different for single inoculation;

(iii) The aggregation is different across all groups .

Details of the maximum likelihood method and the maximum likelihood ratio test are given in Chapter 2. The mean was allowed to vary according to the hypothesis being

tested, increasing the number of estimated parameters. The degrees of freedom used in the maximum likelihood ratio test take this into account.

Table 3.1 Details of the experiments from which data were taken.

Type of *A. suum* infection	Number of pigs	Duration of infection	Dose	Reference
Single dose	32	12 weeks	600 eggs	Petkevičius *et al.* (1995)
Single dose	19	8 weeks	100, 1000 or 10000 eggs	Roepstorff *et al.* (1997)
Single dose	50	8 weeks	600 eggs	Petkevičius *et al.* (1997)
Single dose	36	12 weeks	200 eggs	Eriksen (unpublished)
Trickle inoculation	38	12 weeks	10000 eggs twice weekly	Boes *et al.* (1998)
Trickle inoculation	40	10 - 16 weeks	25 or 500 eggs twice weekly	Eriksen *et al.* (1992b)
Trickle inoculation	12	12 weeks	500 eggs twice weekly	Helwigh *et al.* (1999)
Natural Exposure	15	22 weeks	-	Roepstorff & Murrell (1997)
Natural Exposure	20	20 weeks	-	Petkevičius *et al.* (1996)
Natural Exposure	50	10 weeks	-	Boes *et al.* (1998)
Natural Exposure	50	12 weeks	-	Mejer *et al.* (2000) Thomsen *et al.* (2000) Wendt *et al.* (2000)

3.2.2 RESULTS

Table 3.2 gives the mean intensity and prevalence of infection and the negative binomial aggregation parameter, k, for the three methods of inoculation. All distributions considered did not differ significantly from the negative binomial distribution.

3.2.2.1 Prevalence of Infection

The prevalence of infection in the single inoculation group was significantly smaller ($P<0.001$) than in both the trickle inoculation and the natural exposure group, using both Pearson's χ^2 test and Fisher's exact test. There was no significant difference in prevalence between the trickle inoculation and the natural exposure groups ($P=0.5$).

3.2.2.2 Intensity of Infection

The Kolmogorov-Smirnov test is a non-parametric way of assessing the difference in the ordinal distribution of two samples. The single inoculation group had a significantly lower ranked worm burden than the trickle inoculation group ($P=0.001$) and the natural exposure group ($P<0.001$). There was no difference in the intensity of infection between the trickle inoculation group and the natural exposure group ($P=0.998$).

Table 3.2 The mean and prevalence of infection and the negative binomial aggregation parameter, *k*, calculated for different methods of inoculation

	number of experiments	number of animals	Mean	Prevalence	*k*
Single Dose	4	137	5.8[1]	19.7%[2]	0.05[3]
Trickle Inoculation	3	90	7.7	45.6%	0.16
Natural Exposure	4	135	7.9	50.4%	0.18
Total	11	362	7.1	37.8%	0.12

[1] $P \leq 0.001$ significantly lower than other groups
[2] $P < 0.001$ significantly lower than other groups
[3] $P < 0.0001$ significantly different to other groups, see table 3.3 for explanation

3.2.2.3 Aggregation

Table 3.3 gives the parameter estimates for the three proposed models of aggregation and the p-values for the χ^2 test for model improvement. The model that best describes the data, has two values of *k*. One for the two repeated exposure groups (trickle inoculation and natural exposure) combined and one for the single inoculation group. The single inoculation group had a much higher degree of aggregation than the repeated exposure group.

Table 3.3 The parameter estimates for the negative binomial parameter, k for the three proposed models of aggregation of the data and the p-values for the χ^2 test for model improvement.

Model	Parameter Estimate of k	Log-likelihood	No. of parameters	χ^2 test for model improvements (P-values)
All Exposures	0.12	745.42	2	
Single Inoculation	0.05			
Repeated Exposure	0.17	726.21	4	**<0.0001**
Single Inoculation	0.05			
Trickle Inoculation	0.16			
Natural Exposure	0.18	726.00	6	0.809642

3.2.2.4 Model Prediction

The fit of the predicted negative binomial model to the experimental data, is given in figure 3.1. Natural and trickle inoculated animals had one degree of aggregation and intensity of infection and single inoculated animals another. The model predicted the frequency of each worm burden, given a total population of 362 animals, of which 137 animals had worm burdens distributed according to the negative binomial distribution with a mean of 5.8 and k of 0.05, and 225 animals had worm burdens distributed by the negative binomial distribution with a mean of 7.8 and k of 0.17. The worm burden data from all 362 animals are shown for comparison.

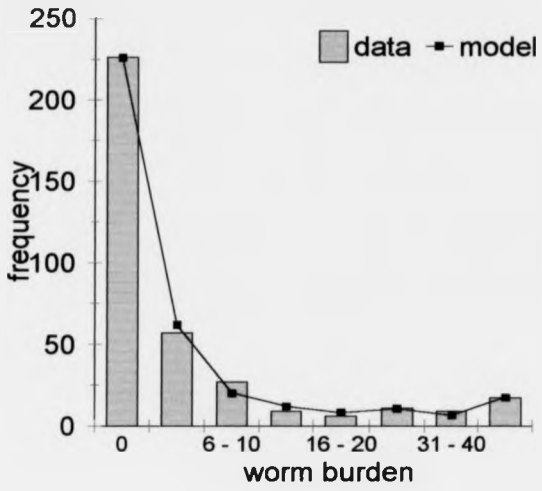


Figure 3.1 The model prediction compared to the data. The data is the frequency of worm burdens from 362 animals, of which 137 received a single inoculation dose and the remaining 225 were repeatedly exposed to infective *A. suum* eggs. The line is the model prediction, given that a proportion of the animals had the expected worm burden distribution following a single dose inoculation and a proportion had the expected worm burden distribution resulting from repeated exposure.

3.2.3 SUMMARY

This investigation has shown that trickle inoculation provides a good model for the prevalence, intensity and aggregation of the worm burden distribution of pigs naturally exposed to *A. suum*. It also demonstrated that repeated exposure to *A. suum* (up to 22 weeks in duration) causes an increase in intensity of infection, prevalence and a reduction in aggregation when compared to a single inoculation. Figure 3.2 shows the worm burden distribution for single inoculation experiments compared to combined natural and trickle inoculation experiments.

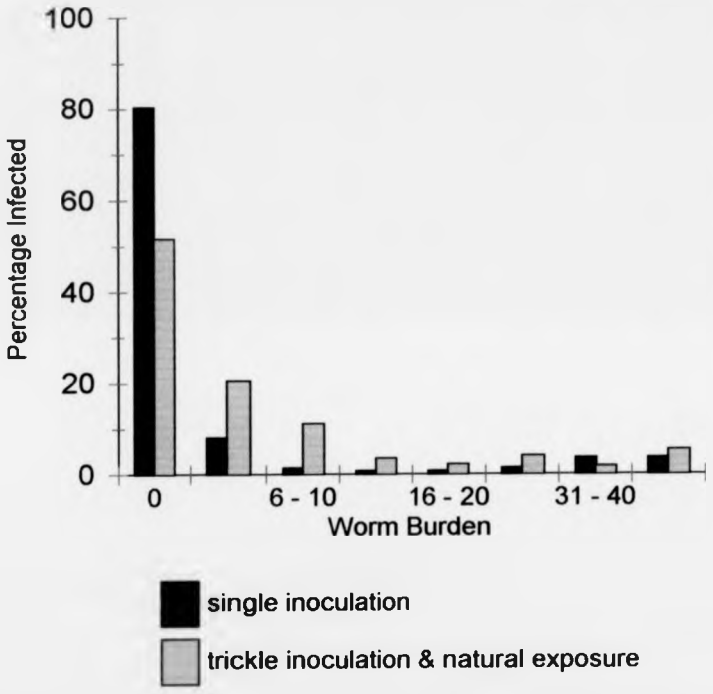


Figure 3.2 The worm burden distribution of single inoculation experiments compared to natural and trickle inoculation experiments combined.

PART II: AN INVESTIGATION INTO THE NUMBER OF PIGS REQUIRED TO OBTAIN ACCURATE INFORMATION ON THE WORM BURDEN DISTRIBUTION

3.3.1 METHOD

The negative binomial distribution was used to model the data (Bliss & Fisher, 1953). This was chosen as it is widely acknowledged to give a good empirical description of over-dispersed discrete data such as the distribution of *A. suum* across a host population (Medley, 1992). The negative binomial distribution is described by the mean intensity of infection, m and the aggregation parameter, k (Anderson & May, 1991). Data sets were simulated using the Monte Carlo method (Hilborn & Mangel, 1997a) and the probability of getting a significant reduction in aggregation on reinfection was estimated for different group sizes.

3.3.1.1. Generating random variables distributed by the negative binomial distribution with aggregation parameter k and mean m.

The Monte Carlo technique generates random variables from a specific distribution. For the negative binomial distribution, the probability $p(w,k,m)$ of a host having w worms given a population with aggregation k and mean m is given by equation 3.1.

$$p(w) = \frac{\Gamma(k+w)}{\Gamma(k)w!}\left(\frac{k}{k+m}\right)^{k}\left(\frac{m}{m+k}\right)^{w} \qquad \textbf{(3.1)}$$

If $p(w,k,m)$ is summed from w=0 to infinity, the sum is 1. The cumulative probability function was compared to a uniformly distributed random number between 0 and 1 to generate a number which represents the number of worms in a host.

The following pseudo-code describes the process by which a random variable is found using the Monte Carlo method:

1. Specify parameters k and m.
   Choose a uniformly distributed random number, U, between 0 and 1.
   Set w=0 and SUM= 0
2. Compute p(w,k,m) from equation 3.1
3. Replace SUM by SUM + p(w,k,m)
4. If SUM $\geq$U, then the current value of w is the number of worms in this host.
   Otherwise replace w by w + 1, and return to step 2.

The real code, as used in Matlab®, is given in Appendix A. This process is illustrated for the negative binomial distribution with k=0.2 and m=6, NB(0.2, 6):

Random number generated: U=0.6458

w=0

p(w,k,m)=0.5032

SUM=0.5032

SUM $\geq$U, set w = w + 1

w = 1

p(w,k,m)=0.0974

SUM=0.6006

SUM ≱U, set w = w + 1

w = 2

p(w,k,m)=0.0565

SUM=0.6571

SUM ≥U; number of worms, w = 2.

This process is repeated for each host in the group.

To demonstrate this, the above distribution NB(0.2,6) was sampled 100 times, representing a group of 100 pigs. The resulting distribution of theoretical worm burdens is shown in figure 3.3. The sample distribution had a mean of 7.2 and a moment estimate of *k* of 0.18.

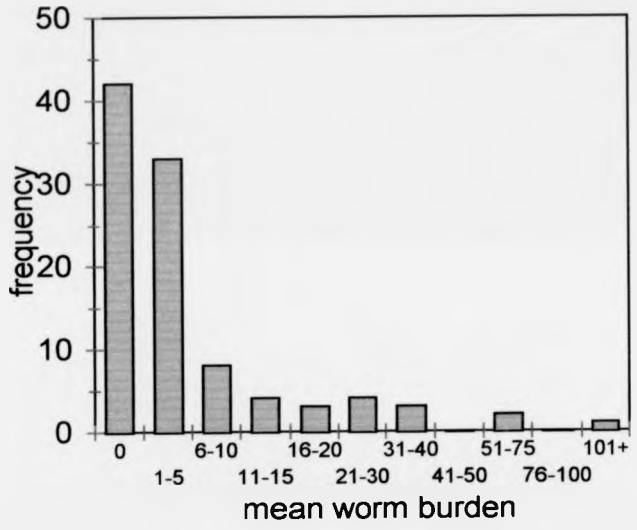


Figure 3.3 The distribution of worm burdens sampled 100 times from a negative binomial distribution with mean of 6 and aggregation parameter of 0.2.

3.3.1.2. A comparison of two sample distributions

Two sample distributions, of a given group size, were generated from the negative binomial distribution described by parameters taken from the natural exposure infection / reinfection experiment (Boes *et al.* 1998). These parameters were chosen, as the objective was to design an experiment which could replicate the results of this experiment, under more controlled conditions.

The first sampled distribution represented the worms obtained at treatment. The second, worms obtained following reinfection. The moment estimate of k and the mean intensity of infection, m, were calculated for each sampled distribution, and the combination of both distributions. The combined distribution was created to represent the null hypothesis that both samples were drawn from the same distribution. The likelihood ratio test was used to assess whether the sampled distributions were best described as two separate distributions or one combined distribution. This was repeated a number of times, for each group size, to ascertain the probability of finding a significant difference between the sampled distributions.

The mean, variance and moment estimate of k were calculated for each distribution using the following three equations, where N is the size of the distribution:

$$m = \left(\frac{1}{N_t}\right)\sum_{i=1}^{N} W \qquad \textbf{(3.2)}$$

$$s^2 = \left(\frac{1}{N-1}\right)\sum_{i=1}^{N}(W - m)^2 \qquad (3.3)$$

$$k = \frac{m^2}{s^2 - m} \qquad (3.4)$$

For each simulation, the difference in log likelihood between the combined distribution, and the initial and reinfection distributions, was calculated. The negative log-likelihood of a worm burden, *w*, coming from a distribution with sample mean *m* and aggregation parameter *k*, summed across all hosts, is given by:

$$L\{w|m,k\} = \sum_{i=1}^{N} -\log\left(\frac{\Gamma(k+w)}{\Gamma(k)w!}\left(\frac{k}{k+m}\right)^{k}\left(\frac{m}{m+k}\right)^{w}\right) \qquad (3.5)$$

where N is the size of the sample distribution.

If twice the difference in log-likelihood was greater than the 5% chi-square value with two degrees of freedom ($\chi^2_{0.05} = 5.99$), the simulation was a success. The total number of successes divided by the number of simulations gave the probability of obtaining a successful result for each group size (the power).

The code used for this procedure is given in the Appendix B.

3.3.2 RESULTS

One hundred simulations were performed on group sizes ranging from ten to forty pigs. This covered the range of logistically and financially feasible group sizes. The number of simulations performed reflected a balance between the time required to perform the simulations and the degree of accuracy obtained. The parameters used to generate the data sets were based on those found by Boes *et al.* (1998). The initial worm burden distribution was generated, by sampling from a distribution with a mean of 10.4 worms per pig, and an aggregation parameter k = 0.09. The reinfection worm burden distribution was generated using a mean of 9.3, and an aggregation parameter, k = 0.69. The probability of obtaining a successful result for different group sizes is shown in figure 3.4.

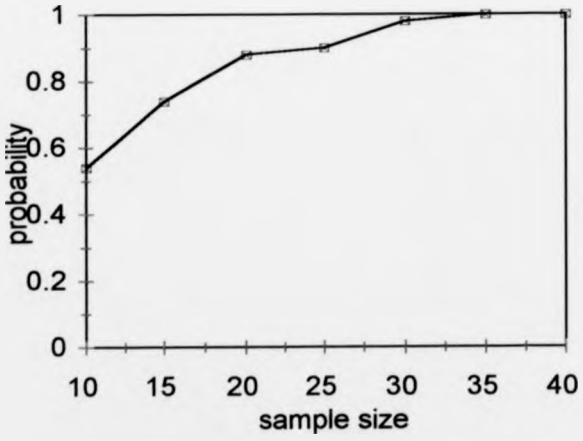


Figure 3.4 The power of a hypothesis, as a function of the number of pigs used in each experimental group

A similar technique was used to assess the probability of obtaining a type I error for different group sizes. The probability of obtaining a statistically significant difference when the two distributions being sampled had the same mean and aggregation parameter k are shown in figure 3.5 for distributions with a mean of m=10 and k=0.09 or k=0.69.

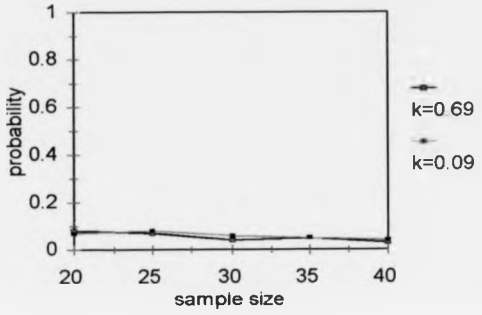


Figure 3.5 The probability of obtaining a statistically different result when the same distribution is sampled twice, as a function of group size

3.3.3 SUMMARY AND DISCUSSION

The probability of getting a successful result using thirty animals, given the stated mean and aggregation parameters, was 98%. The probability of obtaining a statistically significant difference if the same distribution was sampled twice, with thirty animals, was less than approximately 5%. This should equal α (0.05), given a true null hypothesis, however, the small sample sizes increase the chance of type I errors occurring. As the sample size was increased, the probability of a type I error occurring approached α. Another factor to be taken into consideration when interpreting these results is that the negative binomial aggregation parameter, k, was obtained using the moment estimate (for computational reasons). This method is used to obtain an approximation of k and is not as accurate as the maximum likelihood method, which was used when analysing experimental data.

Using more than thirty animals would not greatly improve the chance of obtaining a successful result, or avoiding an incorrect result, but would increase the cost and practical difficulties. To conclude, a group size of thirty animals would be the most cost-effective way to maximise the chance of obtaining a successful result if the results of the natural exposure experiment were correct, and to minimise the chance of obtaining a falsely statistically significant result, if not.

CHAPTER 4: AN EXPERIMENT INVESTIGATING THE DISTRIBUTION OF *ASCARIS SUUM* IN TRICKLE INOCULATED PIGS

4.1 INTRODUCTION

Ascaris suum worm burdens are typically aggregated among the host population, whether they derive from experimental single inoculations, trickle inoculations or naturally from contaminated pasture (Roepstorff *et al.* 1997, Boes *et al.* 1998). In the previous two chapters, it was shown that the extent of the aggregation is dependent upon both the inoculation protocol and, in piglets, the maternal experience of infection. Worm burdens from pigs that were infected either naturally or through trickle inoculation were less aggregated than those from animals that were given a single experimental inoculation. In addition, piglets born to previously infected sows had a less aggregated distribution than piglets born to parasite-naive sows. The longer the duration of exposure experienced by the sows to *A. suum*, the less aggregated the distribution of worms among their piglets became. An experiment studying the reinfection of naturally infected pigs following anthelmintic treatment (Boes *et al.* 1998) also found a reduction in aggregation with experience of infection. However, the change could not be attributed solely to the pigs' immune response, as a number of environmental and behavioural factors may also have been responsible.

The present experimental study was carried out to investigate the hypothesis that a past experience of infection causes a reduction in the aggregation of worms across the host population. The main objective was to replicate the results of the natural exposure

experiment, under controlled conditions, using trickle inoculated pigs. As well as evaluating the pattern of reinfection following treatment, the effects of dose size (number of infective eggs given) on the worm burden distribution was also investigated, where the distribution was characterised by the degree of aggregation, the mean intensity of infection and the prevalence of infection. Other objectives were to describe a possible predisposition of individual pigs to *A. suum*, where behavioural and environmental influences are limited; to examine changes in the fecundity of *A. suum* due to host infection experience; to investigate the formation of a pre-hepatic barrier during a trickle inoculation; and to analyse changes in the rate of establishment.

Due to the highly aggregated nature of the distribution of worms among hosts, the majority of worms are harboured by the minority of hosts (Anderson & Gordon, 1982). This is also true, for the equivalent human infection, *Ascaris lumbricoides* (Anderson, 1985). Morbidity observed in human ascariasis is generally associated with high worm burdens (Pawlowski & Davis, 1989). It has been shown that targeting treatment at the most heavily infected age groups acts disproportionately to reduce the level of infection within a community (Bundy *et al.* 1990, Asaolu *et al.* 1991). If certain individuals were predisposed to heavy infection, selective treatment of these individuals could also have a marked impact on morbidity, though the impact of selective treatment is very much dependent on the mechanisms responsible for the predisposition (Keymer & Pagal, 1990). In additon, Holland *et al.* (1996b) demonstrated that selective treatment programmes were associated with much higher costs. Nonetheless, a predisposition of certain hosts to heavy infection could have important implications both in terms of the

clinical implications and the effect on the transmission dynamics (Anderson & Medley, 1985).

There is considerable evidence for the existence of a predisposition to heavy or light infection in human *A. lumbricoides* infections (Haswell-Elkins *et al.* 1987, Holland *et al.* 1989, Hall *et al.* 1992). Several published studies giving correlation coefficients relating to the association between initial infection worm burdens and reinfection levels following chemotherapeutic treatment indicate that about 35% of individuals retain their relative positions in terms of infection intensity (Bundy & Medley, 1992).Differences in host behaviour, host nutritional status and genetic factors have all been suggested as possible causes (Anderson, 1989). The present experimental design enables predisposition of individual pigs to *A. suum* to be investigated, where exposure to infective stages and host nutritional status have been controlled.

It has been hypothesised that experience of infection may lead to immunological limitations on the parasite, such as a decrease in fecundity (for example Grenfell *et al.* 1995). The treatment-reinfection design of the experiment allows the effect of infection experience on fecundity to be examined. Fecundity has also been inversely associated with the density of parasites within an individual host (Anderson and May, 1991). This hypothesis is also explored using the current experimental data.

The absence of immature worms and liver white-spot lesions, which form in response to the migration of larvae, have been used as evidence of a pre-hepatic barrier to infection induced by chronic natural or experimental infection. Recording the number of liver

white spots provides information on how recently the infection occurred because spots are known to disappear within 4 - 6 weeks of exposure (Eriksen *et al.* 1980). Urban *et al.* (1988) found that pigs that had been naturally exposed to *A. suum* had livers that were notably normal in appearance after challenge, and concluded that a strong intestinal immunity had apparently developed that limited migration of second-stage (sic) larvae from the intestine. Eriksen *et al.* (1992b) examined the response of pigs to repeated inoculations with *A. suum* eggs. Before the patent infection had established, immature worms were present in moderate numbers, with a rough correlation to dose levels, however once adult worms were present, immature worms were scarce. Eriksen *et al.* (1992a) challenged parasite naive and naturally exposed pigs with *A. suum* eggs. The previously exposed pigs had considerably fewer larvae in the lungs compared to naive animals.

The above studies suggest that a long term trickle inoculation would result in an absence of immature worms and white spots. The recent development of an agar gel technique to harvest worms at necropsy (Slotved *et al.* 1997) has enabled much greater accuracy in the collection of immature worms. Using the agar gel technique, Slotved *et al.* (1997) recovered over 99% of larvae found in the small intestine 10 days post inoculation (p.i.), following a single inoculation at two dose levels. One of the aims of the present experiment is to use this technique to reassess the formation of a prehepatic barrier during a long term trickle inoculation.

The changes in the number of animals excreting eggs through time can be used as an indication of when a host first has a sexually mature pair of worms (Jungersen *et al.*

1997). Morphological profiles can also be used to examine epidemiological features (Elkins & Haswell-Elkins, 1989). In particular, comparisons of worm length and biomass profiles across groups may give information on the different rates of establishment, when the start of the exposure period is controlled.

4.2 MATERIALS AND METHODS

4.2.1 Experimental Procedures

Experimental design

Four groups of 30 pigs were trickle inoculated with infective *A. suum* eggs through the feed. Two groups were given a low dose (100 eggs per animal twice weekly) and two groups were given a high dose (6000 eggs per animal twice weekly). All four groups were inoculated for a total of 20 weeks, however, one low dose group and one high dose group were treated with anthelmintics after the first 10 week period. The worm burdens were ascertained at treatment and at necropsy (after 20 weeks of exposure). Table 4.1 gives details of the experimental groups, the dose level that they received, the period of inoculation from which the worm burden resulted, and when the worms were recovered. The duration of the inoculation periods before and after treatment with anthelmintics were chosen so that the results would be comparable to those of the natural infection experiment (Boes *et al.* 1998).

Thirty pigs were used in each group as a simulation study (chapter 3) had shown that there was a 98% chance of obtaining a statistically significant reduction in aggregation, following treatment with anthelmintics, based on the distributions observed in the natural infection / reinfection experiment.

Table 4.1 A summary of the experimental protocol for the four experimental groups. Groups 1 and 3 were trickle inoculated for 10 weeks (A), treated with anthelmintics then trickle inoculated for a further 10 weeks (B). Groups 2 and 4 were trickle inoculated for 20 weeks.

GROUP	DOSE	DURATION	WORMS RECOVERED	TOTAL EXPOSURE
1 A	LOW	10 WEEKS	AT TREATMENT	
1 B	LOW	10 WEEKS	AT NECROPSY	20 WEEKS
2	LOW	20 WEEKS	AT NECROPSY	20 WEEKS
3 A	HIGH	10 WEEKS	AT TREATMENT	
3 B	HIGH	10 WEEKS	AT NECROPSY	20 WEEKS
4	HIGH	20 WEEKS	AT NECROPSY	20 WEEKS

Experimental animals and housing

The experimental groups consisted of parasite-naive Danish Landrace x Yorkshire x Duroc male pigs, aged 5 - 7 weeks at the start of the experiment. The pigs were obtained from a specific pathogen-free (SPF) farm that was surveyed prior to the start of the experiment and found to be free from *A. suum* infection.

The pigs were housed outdoors on a clean pasture and given at least two weeks to acclimatise to the conditions before the experiment began. Once they had begun to excrete *A. suum* eggs, they were moved to clean pasture every three weeks to prevent any additional ingestion of infective eggs. Once in the environment the eggs take approximately 5 weeks to become infective under favourable conditions (Larsen & Roepstorff, 1999). The feed was a traditional diet of ground barley plus supplementary protein, in accordance with a standard feeding regime. Water was available *ad libitum*.

Parasite material

The *A. suum* eggs used for the experimental trickle inoculation doses were collected from female worms obtained from a local slaughterhouse. The eggs were de-coated in NaOCl, then embryonated in 0.1 N H_2SO_4 at room temperature as described previously by Oksanen *et al.* (1990).

Sampling procedure

Faecal samples were taken when the animals were introduced to the pasture and at the start of the infection period to indicate that the animals were not already infected. The animals were also sampled 3 weeks after they had been treated with anthelmintics as an indication that all the worms had been expelled. Faecal samples were taken 10 weeks post-initial-inoculation and 20 weeks post-initial-inoculation (equivalent to 10 weeks-post-treatment in treated groups) for statistical comparison. Furthermore, samples were taken periodically throughout the experiment to monitor the infection status of the groups. The eggs were counted using a modified concentration McMaster technique, which has a lower detection limit of 20 eggs per gram of faeces (Roepstorff and Nansen,

1998). The eggs were distinguishable from the inoculation dose which contained de-coated eggs.

Treatment with anthelmintics

After ten weeks of inoculation, when a patent infection had been established, two groups were removed and treated with 200 mg / kg body weight Piperazine dihydrochloride (Ascarex® Vet., Akzo Nobel) via stomach tube. This was chosen as it is fast acting and easy to administer (water soluble). Fifteen pigs at a time were treated and housed in individual cages. Worm expulsion was recorded for 3½ days, after which the animals were returned to the pasture. The efficacy of the treatment was assessed by post-treatment faecal egg counts.

Faecal matter was sieved using a 1.0 mm mesh sieve and all macroscopically detectable worms were removed and stored in 70% ethanol. A sub-sample was sieved using both a 1.0 mm mesh sieve and a 300µm sieve to assess the number of small worms that may have been missed by the larger mesh. The worm burden for each individual pig was recorded as was the length and sex of each worm, where possible.

Necropsy

All animals were slaughtered after a total of 20 weeks trickle inoculation. Worms were collected from the small intestine using the Agar gel technique (Slotved *et al.* 1997). All worms and larvae were stored in 70% ethanol. The number of worms and larvae found in each pig was recorded as was the length, weight and sex of each worm and the

approximate length of the larvae. The liver texture score and the number of white spots, and the lung pathology were also recorded.

4.2.2 Analytical methods and statistical procedures

The distribution of *A. suum* in pigs is typically over-dispersed and highly aggregated, consequently standard parametric analysis is not appropriate. The non-parametric alternatives to the t-test (to test the difference between groups for independent samples) are usually the Mann-Whitney U test or the Kolmogorov-Smirnov two sample test. The Mann-Whitney U test is the more powerful of the two, however it is incorrect to apply this test when the majority of observations have tied ranks.

The effect of previous experience of infection, and inoculation dose and duration on the mean intensity of infection, the prevalence and the aggregation of the worm burden distribution was examined. First, the difference between the worm burdens at treatment and the worm burdens after reinfection was investigated. Then the relationship between inoculation dose and duration was examined. Finally, the difference in the worm burdens at slaughter between the treated and untreated groups was analysed.

The χ^2 test was used to establish if the negative binomial distribution was a suitable model for the data. The maximum likelihood technique was used to obtain a measure of aggregation of the worm burden distributions, and the likelihood ratio test was used to

test for significant differences (see chapter 2 for details). The χ^2 test was also used to test for significant differences in prevalence of infection between experimental groups.

Reinfection worm burdens were compared to initial worm burdens using a matched pairs signed test. The degree of correlation was examined using Spearman's r_s and Kendall's tau-b rank correlation coefficients. Kendall's tau-b is more appropriate when there are a large number of tied ranks.

Predisposition to light or heavy infection

The predisposition of hosts to light or heavy infection, where the variation in exposure to infective stages and nutritional status had been controlled, was examined. The degree of predisposition between the initial worm burdens and the reinfection worm burdens was investigated using rank correlation coefficients. To allow for comparison with published results, both Spearman's r_s and Kendall's τ_b were calculated, although Kendall's τ_b is more appropriate when there are a large number of tied values.

The correlation between the number of immature worms and the number of adult worms found in each host at necropsy was also examined. Worm burdens obtained as a result of anthelmintic treatment were not used due to the lower accuracy in the harvest of immature worms. Again, the level of significance was tested using both Spearman's r_s and Kendall's τ_b correlation coefficients.

Fecundity

The fecundity of each group was calculated as the sum of the eggs per gram of faeces (EPG) divided by the total number of adult female worms. To investigate the hypothesis that infection experience may result in immunological limitations on parasite reproduction, the fecundity of female worms after 10 weeks reinfection was compared to the fecundity after the initial 10 week period of inoculation. The fecundity of each pig was calculated by dividing the EPG by the number of adult female worms, where both the EPG and the number of females were greater than zero. Differences between the fecundity after reinfection and the initial fecundity observed at the time of treatment were tested using the Kolmogorov-Smirnov test. The average fecundity of each group was also examined visually, by plotting the initial and reinfection EPG data against the number of female worms, and comparing the gradients of the best linear fits.

To investigate the hypothesis that fecundity is inversely associated with the density of parasites within an individual host, the fecundity was calculated for the 20 weeks trickle inoculation low dose and high dose worm burdens (groups 2 and 4) and plotted against the total worm burden, for EPG values and worm burdens greater than zero. These groups were chosen as they had the longest period of infection and were thus most likely to reveal an effect.

Prehepatic barrier

The number of white spots, the degree of liver fibrosis and the number of immature worms obtained at necropsy were compared across the two dose groups to see first, if a prehepatic barrier existed, and second, to assess the relative strength across the two doses. Immature worms that were recovered were divided into those that were smaller or approximately equal to 5 mm, and those that were greater than 5 mm. This length was chosen as it is the approximate length of immature worms at 14 days p.i. (measured at DCEP). After 14 days p.i. the majority of immature worms are expelled, resulting in a small over-dispersed worm burden by 28 days p.i. (Roepstorff *et al.* 1997). By comparing the number of immature worms recovered that are smaller than 5mm, the effect of dose on the pre-expulsion worm burdens was examined.

Establishment rates

The effect of dose on the rate of establishment of patent infections across groups of animals was investigated by examining changes in the coproprevalence though time. When calculating coproprevalence, a cut-off point of 200 EPG (as suggested by Roepstorff 1997) was used to exclude false-positive animals.

The rate of establishment of worms within the hosts of different groups was examined inferentially using the worm length and biomass distributions. This was based on the premise that as the worms mature, they increase in weight and length. The relationship between weight and length was examined in male and female worms. The mean biomass for each dose after 20 weeks trickle inoculation was compared by plotting the total biomass of worms within each host against the worm burden, and examining the

gradient. This was also used to explore the hypothesis that there may be density dependent limitations on growth. The correlation between the biomass and the worm burden was assessed using Spearman's r_s correlation coefficient.

Comparable establishment between the two doses was also examined by plotting the cumulative frequency distribution of decreasing worm lengths. Again, based on the assumption that length increases with age and thus reflects an earlier establishment. When examining the cumulative length distributions it is important to note that the worms will grow to a maximum length, thus over time there will be a build up of large worms. When this happens, the left hand side of the graph will represent a range of establishment times, dependent on the duration of the infection. Given enough time, the mature worms will also die. Secondly, it is not known whether age and length have a linear relationship, so we can not assume that establishment time runs linearly along the x-axis, just that a longer worm is likely to be older by some unknown amount.

4.3 RESULTS

Full results are given in Appendix C. A summary of the results, by group, are given in table 4.2.

A total of five animals were removed from the experimental groups during the course of the experiment. Egg doses were adjusted accordingly. None of the deaths were related to the *Ascaris* infection.

All animals tested negative for the presence of *A. suum* eggs in their faeces, when they were placed on the pasture. All animals that were treated with anthelmintics 10 weeks after their first inoculation tested negative when faecal samples were taken for analysis three weeks after treatment.

Both the high dose and the low dose groups began to excrete eggs 6.5 weeks after their first inoculation. In order to accommodate the animals during the week of anthelmintic treatment, the start of inoculation in the high dose groups was delayed by one week. Consequently, the high dose groups had three weeks to acclimatise to the pasture, and were present on the pasture for a total of 9.5 weeks before they began to excrete eggs. As the prepatent period for *A. suum* is between 6 and 8 weeks, it can be concluded that the pasture was clean when the animals were placed there.

4.3.1 *Post Mortem Observations*

Lung pathology was normal in all animals except one pig in group 4 (high dose, 20 weeks inoculation, no anthelmintic treatment) in which a few granulomas were observed: 41 immature worms and 6 adult worms were found in this animal at necropsy. Moderate liver fibrosis was observed in 11% of the low dose group and 27% of the high dose group, all other livers were normal. The number of white spots observed is discussed in detail in section 4.3.5.

4.3.2 *The Worm Burden Distributions*

The distribution of worms across hosts for each trickle inoculated group is shown in figure 4.1. The parameters associated with these distributions are given in table 4.2. The χ^2 test was used to test whether there was any difference between the observed distribution of worms in each group and the expected theoretical negative binomial distribution, given the parameters. The null hypothesis, that the negative binomial distribution was a suitable model for the data, was accepted in all cases.

Table 4.2 Summary of the experimental results for each group of trickle inoculated pigs. Parasitological and pathological findings and the worm burden distribution parameters.

GROUP	Trickle Inoculation Dose [a]	Number of hosts in group, n	Trickle Inoculation period (weeks)	Body weight (kg)	Mean worm burden of group, M	Prevalence of infection in group (%)	Negative Binomial parameter, *k*	White spots[b]	Sex ratio [c] (♀:♂)	Fecundity [d]
1 A	100	29	10	43.4	8.03	65.5	0.38	-	1:0.3	508.4
1 B	100	29	10	95.4	2.69	48.3	0.28	2.5	1:0.5	132.4
2	100	29	20	90.7	9.55	72.4	0.39	4.4	1:0.7	327.0
3 A	6000	29	10	47.5	3.55	51.7	0.26	-	1:0.5	267.3
3 B	6000	28	10	104.8	4.32	53.6	0.27	3.2	1:0.8	246.7
4	6000	29	20	90.8	17.38	69.0	0.28	15.9	1:0.5	202.7

[a] Number of eggs per animal, twice weekly
[b] Lymphonodular and diffuse hepatic white spots
[c] Sex ratio calculated as the ratio Σ♀: Σ♂ for each group
[d] Fecundity calculated as ΣEPG /Σ♀ for each group

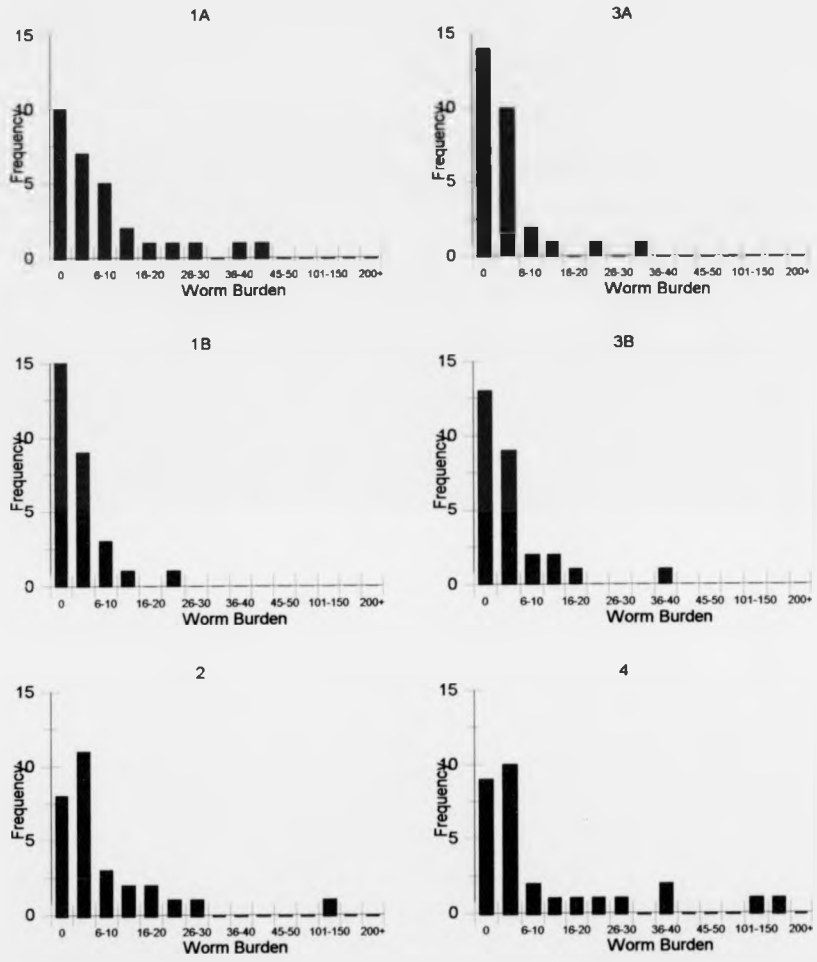


Figure 4.1 The distribution of *A. suum* in experimentally infected animals. Groups 1 and 3 were treated with anthelmintics (A=worm burdens at treatment, B=reinfection worm burdens at necropsy). Groups 2 and 4 were not given anthelmintics

Figure 4.1 suggests that after the initial 10 weeks trickle inoculation (groups 1A and 3A), an animal in the low dose group had a higher chance of being infected with *A. suum*, and was likely to have a higher worm burden, than an animal in the high dose group. Reinfection after a further 10 weeks trickle inoculation, following treatment with anthelmintics, seems to have resulted in a lower prevalence in the low dose group than it initially had, and a marginally higher prevalence in the high dose group. Comparatively, the reinfection distributions are similar in both dose groups. Finally, after 20 weeks uninterrupted trickle inoculation, both dose groups had a higher prevalence and mean than at any other time.

In the present study we found no evidence of past experience of infection causing a reduction in the aggregation of the worm burden. In the low dose group, aggregation actually increased on reinfection, though not significantly. In the high dose group there was no noticeable difference between the degree of aggregation in the initial infection and the reinfection worm burdens. When comparing the initial infection with the reinfection worm burdens, it was also found that there was no significant difference in the prevalence of infection, in either group. A matched pairs sign test revealed that the mean intensity of the initial worm burden was significantly greater than the reinfection worm burden in the low dose group ($p=0.006$). Whilst, in the high dose group, no significant difference was found between the mean intensity of infection of the initial and reinfection worm burdens.

Setting aside the reinfection worm burdens, the relationship between inoculation dose and duration, and their effect on the worm burden distribution was examined. There

appears to be a trend for aggregation to increase (k decreases) with increasing dose level; inoculation duration had no noticeable effect. In the low dose groups, $k = 0.38$ and 0.39 after trickle inoculation for 10 weeks and 20 weeks respectively. In the high dose groups, k was reduced to 0.26 and 0.28 after trickle inoculation for 10 weeks and 20 weeks respectively. There is also a trend for a decrease in prevalence with increasing dose. After 10 weeks trickle inoculation the prevalence was 65.5% in the low dose group and 51.7% in the high dose group. After 20 weeks trickle inoculation, prevalence was 72.4% in the low dose group and 69.0% in the high dose group. An increase in both the prevalence and mean intensity of infection was associated with an increase in the inoculation duration, though this was more pronounced in the high dose group (mean intensity of 3.6 at 10 weeks increasing to 17.4 at 20 weeks).

The effect of treatment with anthelmintics on the worm burdens was also examined by comparing worm burdens obtained at slaughter between the treated groups (1B and 3B) and the untreated groups (2 and 4). When the results of both dose groups were combined, the treated animals had a statistically significant lower prevalence and intensity of infection than the untreated groups, see table 4.3 and figure 4.2. This could be due to the difference in the period of inoculation, as a trend for increasing prevalence and mean intensity of infection with increased inoculation duration was observed when comparing the worm burdens obtained at treatment with the 20 weeks inoculation worm burdens. However the difference when comparing the 20 weeks trickle inoculation worm burdens to the 10 weeks reinfection worm burdens is greater, and statistically significant, which may be due to the added effect of past experience of infection on the reinfection worm burdens.

The degree of aggregation of the two distributions was also significantly different, the treated group being more aggregated (see table 4.3), though the analysis was strongly influenced by the intensity of infection.

The significance of the difference in mean intensity of infection was found using the Kolmogorov-Smirnov test. The χ^2 test was used to find the significance of the difference in prevalence. The p-value for the negative binomial parameter, k was obtained from a likelihood ratio test, for the worm burdens being best described by two negative binomial distributions (treated and untreated) rather than one. This analysis was strongly influenced by the difference in the mean worm burdens and the combined mean worm burden of 8.5.

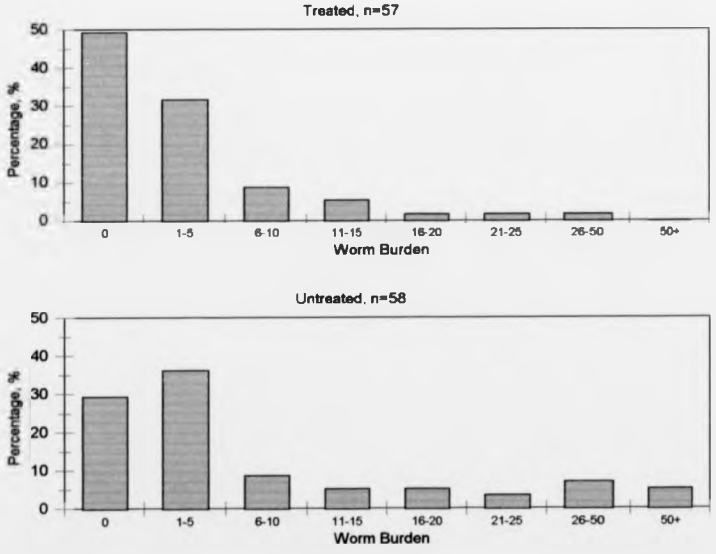


Figure 4.2 Distribution of worm burdens after 20 weeks exposure

Table 4.3 The difference in mean intensity of infection and the prevalence of infection between the worm burdens of the animals treated with anthelmintics at week 10 and the untreated animals, after 20 weeks exposure

	TREATED 1B & 3B	UNTREATED 2 & 4	*p* value
Mean	3.5	13.5	0.024
Prevalence	50.9%	70.7%	0.029
Negative Binomial parameter, k	0.27	0.31	0.037

4.3.3 Predisposition to heavy or light infection

A statistically significant level of association was observed between initial and reinfection worm burdens, Spearman's $r_s = 0.37$ (p=0.004) and Kendall's $\tau_b = 0.29$ (p=0.007), suggesting that individual hosts were predisposed to reacquire a light or heavy infection. To allow for comparison with published results, both Spearman's r_s and Kendall's τ_b were calculated, though for this data especially, Kendall's τ_b is more appropriate as it takes into consideration the large number of tied values, which were mostly due to uninfected animals. The correlation coefficients found in this experiment, show close agreement with those found by Boes *et al.* (1998) when studying the natural infection and reinfection of pigs with *A. suum* in an experiment of the same duration, in which Spearman's $r_s = 0.39$ and Kendall's $\tau_b = 0.31$.

To allow for further comparison with the results of Boes *et al.* (1998), Table 4.4 was produced in which the mean worm burden at treatment and necropsy are shown for

groups classified according to the level of worm burden recovered at treatment: (i) no worms, (ii) low worm burdens (less than the mean recovery), (iii) high worm burden (greater than the mean recovery). Pigs with high worm burdens at treatment had a lower mean on reinfection, but overall, the pattern of infection intensity was repeated at necropsy.

Table 4.4 *Ascaris suum* burdens after reinfection in trickle inoculated pigs 20 weeks post-initial inoculation, classified by initial worm burden recovered at anthelmintic treatment (10 weeks post-initial inoculation), after Boes *et al.* 1998.

Intensity category		Worm burden at treatment	Worm burden after reinfection
	N	Mean	Mean
No worms	23	0	1.6
Low burden (<6.4)*	17	2.4	2.8
High burden (>6.4)	17	19.2	6.8

* A mean worm burden of 6.4 worms was recovered at anthelmintic treatment for the combined low dose and high dose treated groups.

Further support for a predisposition comes from the positive association observed between the number of immature worms and adult worms found at necropsy. The correlation was significant, Spearman's $r_s = 0.36$ (p=0.0001) and Kendall's $\tau_b = 0.29$ (p=0.0001), indicating that animals with high adult worm burdens were also likely to harbour a high number of immature worms.

4.3.4 Ascaris suum Fecundity

Experience of infection

A Kolmogorov-Smirnov test revealed a statistically significant difference between the initial and reinfection female worm fecundities (p=0.0014). Figure 4.3 shows the initial and reinfection EPG against the number of female worms recorded at that time, for each pig in the treated groups. The gradient of the best fit line represents the average fecundity of the group, thus the lower gradient of the reinfection data indicates a lower fecundity. It can be seen from the figure that infection experience was associated with a reduction in fecundity, though this could be due to a delay in reinfection or a prolonged period of migration as well as immunological limitations on the parasite reproduction.

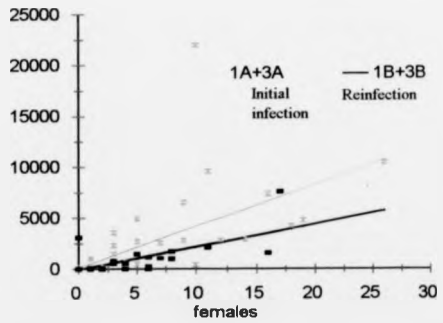


Figure 4.3 The relationship between egg output and number of female parasites per host, and the effect of infection experience on fecundity

Density-dependent constraints

Figure 4.4 shows the fecundity plotted against the total worm burden for the low dose and high dose 20 weeks trickle inoculation groups. It appears that there may be a trend for fecundity to decrease with increasing worm burden, however, the sample size is too small to reveal a definite effect and the epidemic state of infection means that there is a large spread in the fecundity due to newly acquired infection. The analysis is better performed under endemic conditions, when the egg output per female can be expected to be more stable. The graph also indicates that force of infection (dose level) does not appear to affect the level of fecundity

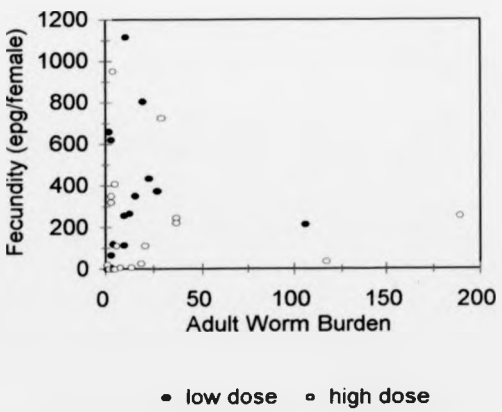


Figure 4.4 The relationship between fecundity and adult worm burden after 20 weeks trickle inoculation at two dose levels

4.3.5 *Prehepatic Barrier*

Using the agar gel technique it was possible to recover larvae as small as 2.0 mm, which is the approximate size of the larvae 10 days p.i. (measured at DCEP), when they return to the small intestine post-migration (Roepstorff *et al.* 1997). The mean number of immature worms recovered and the mean number of liver white spots observed following 20 weeks trickle inoculation are shown as a function of dose in figure 4.5.

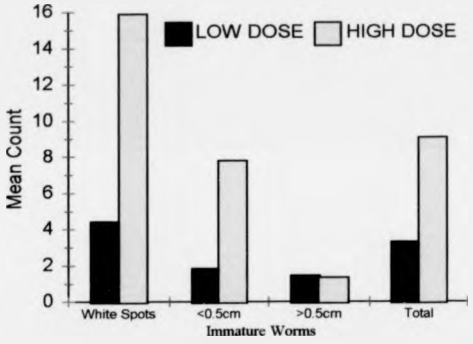


Figure 4.5 The mean number of white spots and immature worms recovered after 20 weeks trickle inoculation at two dose levels

This figure suggests that although there may be a drastic reduction in the number of larvae and white spots observed after the first larval cohort has migrated, any pre-hepatic barrier is by no means absolute. In addition, the number of larvae migrating, as indicated by the number white spots and larvae <0.5cm, are roughly correlated with dose level. A significantly higher prevalence of moderate liver fibrosis found in the high dose group (χ^2 test, $p<0.05$). Moderate liver fibrosis was observed in 27% of animals in the high dose group compared to only 9% of animals in the low dose group, all other animals had

normal livers. The number of immature worms recovered from the small intestine became indistinguishable by dose when they were greater than 0.5 mm in length.

4.3.6 Rates of Establishment

The effect of dose on the rate of establishment of patent infections was investigated. Figure 4.6 shows the changes in the percentage of animals excreting eggs through time for both dose groups. A cut-off point of 200 EPG was used to exclude false-positive animals. The figure clearly shows a much faster rate of establishment in the low dose group than the high dose group. It does however only apply to the prevalence, not the overall establishment.

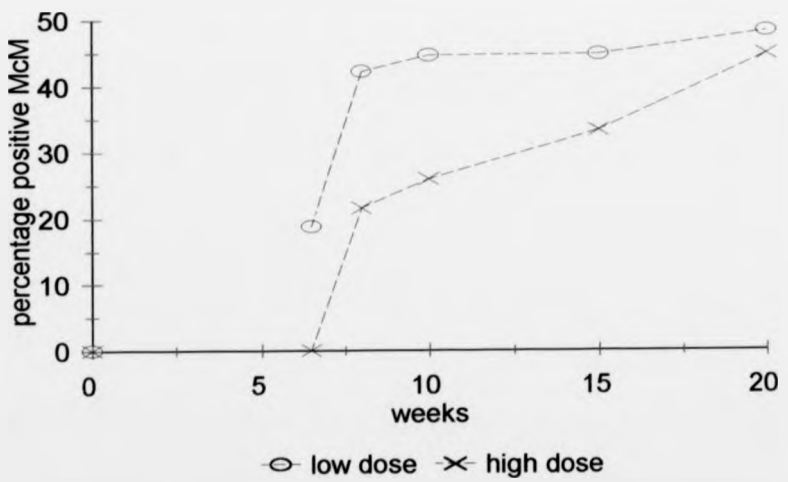


Figure 4.6 The change in prevalence of pigs excreting *A. suum* eggs through time

As might be expected, the weight of a worm increases exponentially as a function of it's length, see figure 4.7. There is no difference between the weight of male and female worms at any given length.

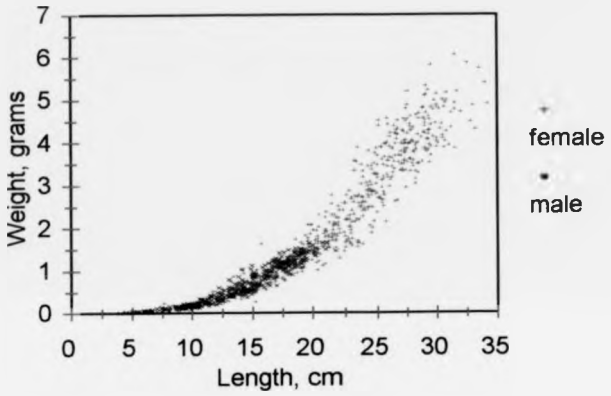


Figure 4.7 The relationship between weight and length for male and female *A. suum* worms

This relationship can be used to analyse changes in the rate of establishment with respect to the average worm weight across dose levels. Figure 4.8 shows the biomass against the worm burden for the two dose levels.

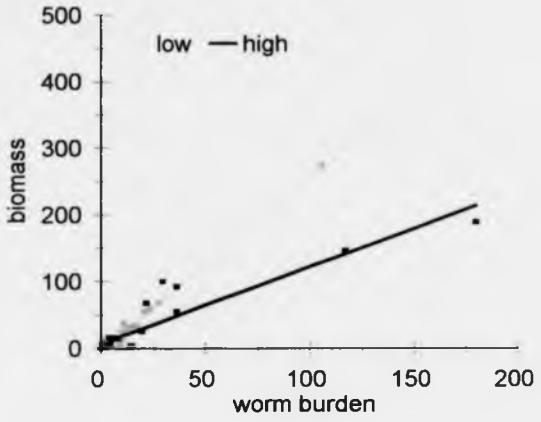


Figure 4.8 Biomass against worm burden after 20 weeks trickle inoculation at two dose levels

At both dose levels, there was a significant linear relationship between biomass and worm burden (Spearman's r= 0.9635 for the low dose group and r=0.9738 for the high dose group, p<0.0001 for both groups), indicating the absence of density dependent limitations on the growth of successfully established parasites. This has also been observed in the comparative human infection *A. lumbricoides* (Elkins & Haswell-Elkins, 1989). There was also an increase in the average weight of worms in the low dose group (2.34 g) compared to the high dose group (1.56 g). This may be due to three things. Firstly, if the worms established earlier in the low dose group, they would on average be older, and consequently there would be a higher average weight in the group. Secondly, if there was continual establishment in the high dose group but no new establishment in the low dose group, the average weight in the high dose group would be biassed towards young worms. Finally, the difference in weights could be explained by suppression of growth in the high dose group, due to host factors (such as immune response).

The comparable establishment through time between the two doses was examined for both female and male worms by plotting both the length frequency distribution (figure 4.9) and the cumulative frequency of worm lengths (figure 4.10), based upon the assumption that length increases with age and reflects an earlier establishment.

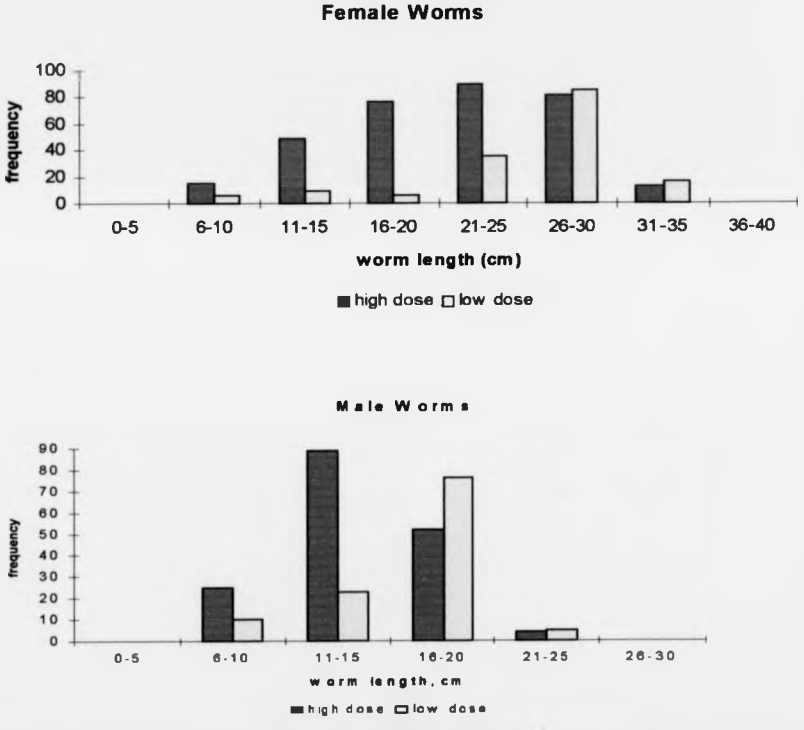
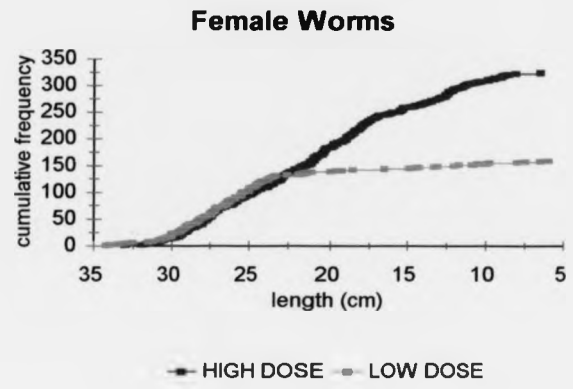


Figure 4.9 The length frequency distributions of (a) female and (b) male *A. suum* worms found at necropsy after 20 weeks trickle inoculation as a function of dose level. Both groups contain 29 animals. The high dose group was trickle inoculated with 6000 eggs per animal twice weekly. The low dose group was trickle inoculated with 100 eggs per animal twice weekly.



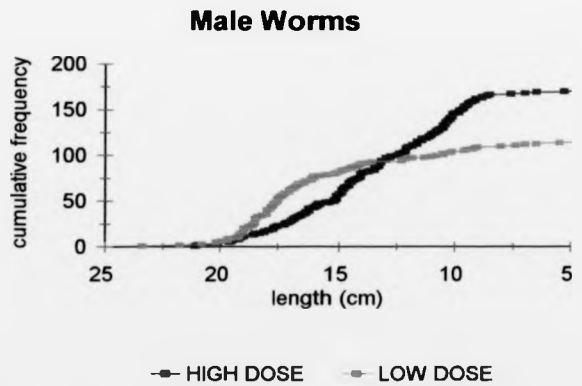


Figure 4.10 The cumulative frequency distribution of the lengths of female and male worms after 20 weeks trickle inoculation at two dose levels.

The rate of establishment of the female worms was initially the same in both dose groups. Subsequently, establishment became much greater in the high dose group compared to the low dose group. The male worms established more quickly in the low dose group than the high dose group, which might explain the difference in egg excretion early on in the infection. Again the establishment of male worms in the low dose group subsequently became reduced compared to the high dose group.

The cumulative frequency distribution of length suggests that the higher mean biomass in the low dose group, is partly due to the larger number of small worms (male and female) in the high dose group and partly due to the number of large male worms in the low dose. The larger males in the low dose group are likely to reflect the earlier establishment of males in this group, as suggested by changes in EPG through time, though it may also be due to an immunological suppression of growth in the high dose group.

4.4 SUMMARY AND DISCUSSION

One of the primary aims of this experiment was to explore whether experience of infection caused a reduction in the aggregation of the worm burden distribution. In the natural exposure experiment (Boes *et al.* 1998), reinfection after anthelmintic treatment resulted in a significant reduction in aggregation. In the present experiment, the force of infection was controlled, using a trickle inoculation, so that any change in aggregation could not be attributed to possible changes in the number of infective eggs available. The

results of the present study demonstrate that previous experience of infection does not cause a reduction in the aggregation of the worm burden distributions when pigs are trickle inoculated with either 100 or 6000 infective eggs twice weekly. The disparity between these results and those of the Boes *et al.* (1998) could be due to a difference in the force of infection either within the natural exposure experiment (between the initial infection period and the reinfection period) and / or between the natural exposure experiment and the present trickle inoculation experiment. More specifically, the reduction in aggregation observed in the natural exposure experiment may have been caused by a change in the number of infective eggs on the pasture due to environmental factors, for example if the weather became more favourable to the embryonation of eggs on the pasture. Equally a change in host behaviour, such as rooting, with age may have been responsible. A combination of these factors may also explain the results; the microclimate of the eggs may have been altered through time by the presence of the pigs on the pasture as a result of their rooting behaviour. In addition to this, the infection of the pigs during the first 10 week period would have altered the infectivity of the pasture during the second 10 week period, especially as the anthelmintic treatment was not 100% successful. It is also possible that if the force of infection in the natural exposure experiment differed greatly from those used in the present studies, the dynamics of infection may be altered. This hypothesis is explored further in Chapter 5 and discussed fully in Chapter 6.

The effect of the duration of the inoculation period and the trickle inoculation dose level on the degree of aggregation in the worm burden distribution were also investigated. The duration of the inoculation period (in absence of anthelmintic intervention) had no effect

on aggregation. It is possible that higher doses may result in a higher degree of aggregation, however the sample sizes used in this experiment were not large enough to detect a statistically significant difference.

Experience of infection was associated with a reduction in fecundity of the female worms, which may indicate an immunological suppression of parasite reproduction or a delay in reinfection after treatment. There was no evidence of density dependent constraints on fecundity, though these may have become more apparent with time.

The significant correlation between the reinfection worm burdens and the initial worm burdens suggests that animals are predisposed to light or heavy infection. As the host nutritional status and the distribution of infective stages were controlled, it is likely that the observed predisposition was a result of genetic factors. Further support comes from the significant correlation that was observed between the number of immature worms and the number of adult worms found at necropsy. This suggests that either animals that harbour a heavy worm burden may be more susceptible to the migration of new larvae, or that the animals which are most susceptible to migrating larvae develop heavy worm burdens.

The number of white spots recorded after 20 weeks inoculation was roughly correlated with dose size. Moreover the number of larvae returning to the small intestine post-migration was also dependent upon the size of the inoculation dose. However the number of larvae found in the small intestine that was larger than 0.5 cm were indistinguishable by dose. A similar effect has been observed following a single

experimental inoculation at three doses (Roepstorff *et al.* 1997) where the number of immature worms recovered from the intestine reflected the dose given up to day 14 p.i. At day 17 p.i. there was no significant difference in the recovery between groups. Even though a pre-hepatic barrier may develop following repeated inoculations, it is by no means absolute and the mechanics of infection are similar to those of a single infection, just at a diminished level.

The most noticeable effect of dose was in the change in prevalence of animals excreting eggs through the course of the infection. It appears that animals in the low dose group became infected with a mated pair of worms much more quickly than animals in the high dose group, which had a steady but gradual increase up to the level of the low dose group by the end of the 20 weeks inoculation. There was very little increase in the number of animals in the low dose group excreting eggs after the initial rise. Examination of the worm lengths supports this. The high dose group had many more small worms than the low dose group. The number of large females was similar in both dose groups, but there were many more large males in the low dose group. This suggests that although the low dose group was initially more susceptible to the establishment of patent infection than the high dose group, the infection was more persistent in the high dose group.

CHAPTER 5: A DYNAMIC MODEL OF COPROPREVALENCE

5.1 INTRODUCTION

Mathematical models describing the transmission of infectious diseases can usually be categorised as either prevalence models or density models (Dietz, 1982). Previous models investigating the dynamics of helminth infections have tended to fall into the latter class, in which changes in the parasite intensity at different stages of the life cycle are modelled, using the parasite immigration and death rates within the host. For a review of gastrointestinal nematode models, see Anderson (1987) and Smith & Grenfell (1994). In this chapter a prevalence model is developed to investigate the dynamics of infection. The change in prevalence of infection across the host population is modelled, based on the results from the experimental trickle inoculation described in Chapter 4, which revealed a change in coproprevalence as a function of inoculation dose through time.

Contradicting results were found between a natural infection experiment (Boes *et al.* 1998) and the trickle inoculation experiment (described in the previous chapter) when comparing the change in aggregation following anthelmintic treatment. One hypothesis that may explain the disparity, is that the force of infection was markedly different from those used in the trickle inoculation experiment. As the model generated in this chapter explores the relationship between force of infection and prevalence it may be possible to estimate the equivalent twice weekly dose encountered by the naturally infected animals. The aim being to assess whether the differences in aggregation between the natural

infection experiment and the trickle inoculation experiment before and after treatment could be explained by differences in force of infection.

5.2 THE MODEL

A simple model was constructed to represent the change in coproprevalence through time as a function of twice-weekly trickle dose level, as described in the previous chapter. Assumptions of the model are as follows:

- Animals are only exposed to the trickle inoculation dose and as a consequence the number of infectious animals in the population does not affect the force of infection.
- Prior to the first inoculation dose, all animals have no experience of infection.
- The birth rate and mortality rate of the host population do not affect the dynamics of the model.
- The duration of infection is greater than the time scale of the model, so that once an animal becomes infected it does not recover.
- All animals in the host population may become infected. The maximum attainable prevalence (coproprevalence) is 100%, and no animals are immune at the start of the model.
- A latent period between infection and infectiousness is included to represent the period during which the larvae migrate and mature into adult worms. As an infected animal will, after the latent period, become infectious, the term "infected" does not include animals that harbour single sex or single worm infections.

From the data in Chapter 4 (see figure 4.6), it can be seen that the two most important features of the model are:

1. The prevalence of infection when the animals first become infectious is inversely associated with the dose level.

2. The rate of increase in prevalence through time is positively associated with the dose level.

In the experiment the initial prevalence is zero. In the model, the initial proportion of infected animals has a different interpretation and represents the proportion of animals that become "infected" at the first inoculation dose (time zero) and will (after the latent period) become infectious. The initial proportion of animals that are infected, Io, and the rate of change, β, are both functions of the inoculation dose:

$$I_0 = f\{Dose\} \qquad \beta = f\{Dose\} \tag{5.1}$$

The number of infected individuals at time t, I(t) is modelled with an asymptotic function, chosen to give a maximum prevalence of 100%:

$$I(t) = I_0 + U_0\left(1 - e^{-\alpha t}\right) \tag{5.2}$$

Where U_0, equivalent to $1 - I_0$, is the number of animals that remain "uninfected" after the first inoculation dose and α is a parameter.

The derivative of this expression gives the subsequent rate of infection, β:

$$\frac{dI}{dt} = aU_0\left(1 - e^{-\alpha t}\right) = \beta \qquad \textbf{(5.3)}$$

For β to increase with dose, the parameter α must also increase as a function of dose. Consequently we set α as a simple linear function of dose:

$$\alpha = \alpha_1 D + \alpha_2 \qquad \textbf{(5.4)}$$

where the parameters α_1 and α_2 are estimated, and D is dose given as number of eggs per host (twice weekly).

The expression for Io was obtained from a comparison of the initial coproprevalence observed in the trickle inoculation experiment with the relationship between dose level and prevalence of infection following a single inoculation. This is described in a later section. First, the relationship between dose and prevalence following a single inoculation is explored.

A compartmental diagram of the full model is shown in figure 5.1. Because of the assumptions stated above for the simple model, loss of infection, ν, loss of immunity, γ, and number of immune animals, R, are all set to zero. They are included in the diagram as they are discussed later in the chapter.

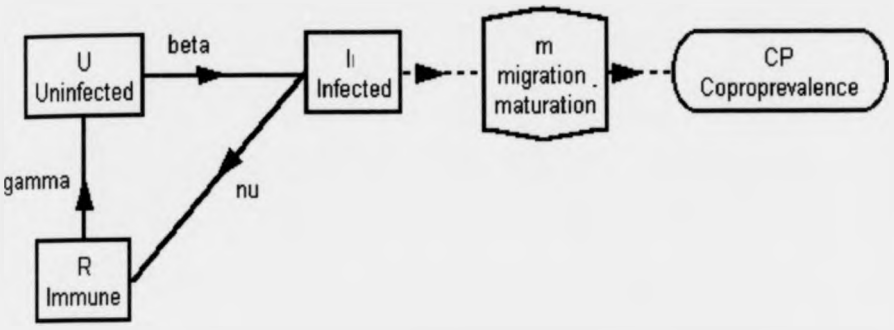


Figure 5.1 A compartmental diagram of the model for coproprevalence. The initial exploration of the model is concerned solely with the rate at which uninfected hosts become infected, consequently ν, γ and R are set to zero. The model assumes that prior to the first inoculation at time zero, all animals are uninfected. A latent period between becoming infected, I, and becoming infectious (to the environment) is represented by *m*. As the final output of the model is coproprevalence, CP, the term infected only applies to animals that will, after the latent period, harbour a mated pair of worms.

Prevalence as a function of dose following a single inoculation

In early studies, in which pigs were inoculated with varying numbers of infective eggs, an inverse relationship between dose level and adult worm burden was observed (Roneus, 1971; Andersen *et al.* 1973; Jørgensen *et al.* 1975). The initial study by Roneus (1971) included only 1 pig per dose level, and the subsequent investigations by Andersen *et al.* (1973) and Jørgensen *et al.* (1975) were limited to 3 groups of 3 pigs, and 2 groups of 5 pigs respectively. Consequently it was not possible to identify an effect of the number of infective eggs given on the prevalence of infection.

More recently, Roepstorff *et al.* (1997) infected three groups of 52 pigs with 100, 1000 or 10000 infective eggs. Although animals were removed throughout the experiment to

study the migratory phase, the infection was allowed to reach patency (6 - 8 weeks pi) in at least ten animals in each group. We have used this data in figure 5.2 to demonstrate that worm burden prevalence is a function of the number of infective eggs given in a single inoculation. The 90% confidence intervals are shown.

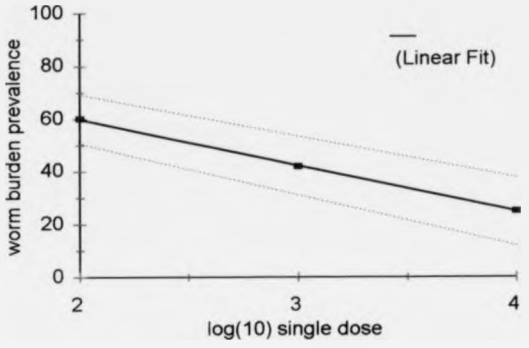


Figure 5.2 Worm burden prevalence as a function of log(10) dose, following a single inoculation

Figure 5.2 shows that there is a significant inverse relationship between prevalence and $\log_{10}$ dose ($p=0.0105$). Of the variation in prevalence, 99.95% is explained by variation in $\log_{10}$ dose. The regression equation describing the relationship between prevalence and dose is:

$$prevalence = 95 - 18\log_{10}(dose) \qquad \textbf{(5.5)}$$

This suggests that the maximum attainable prevalence following a single dose inoculation is 95%, however after repeated inoculations this may rise to 100%, so the assumption given earlier of a maximum attainable prevalence of 100% still holds.

A comparison of prevalence following a single inoculation with coproprevalence during a trickle inoculation

The relationship described above was obtained to represent the proportion of animals, I_0, that become "infected" after the first inoculation dose. Before inclusion into the model that is being constructed, we need to assess how well an equation derived from a single experimental inoculation fits data obtained from a trickle inoculation experiment. To do this, the regression equation (eqn. 5.5) was used to predict the prevalence that would be expected given a single dose of 100 or 6000 eggs per animal, which were the doses given to the animals in the trickle inoculation experiment. The predicted, or "expected", prevalences were compared to the observed coproprevalences from the trickle inoculation experiment, as described in Chapter 4. The ratio between observed and expected prevalence through time is shown for both dose groups in figure 5.3. The ratio through time is shown to illustrate the latent period, in addition to demonstrating the differences resulting from inoculation protocol.

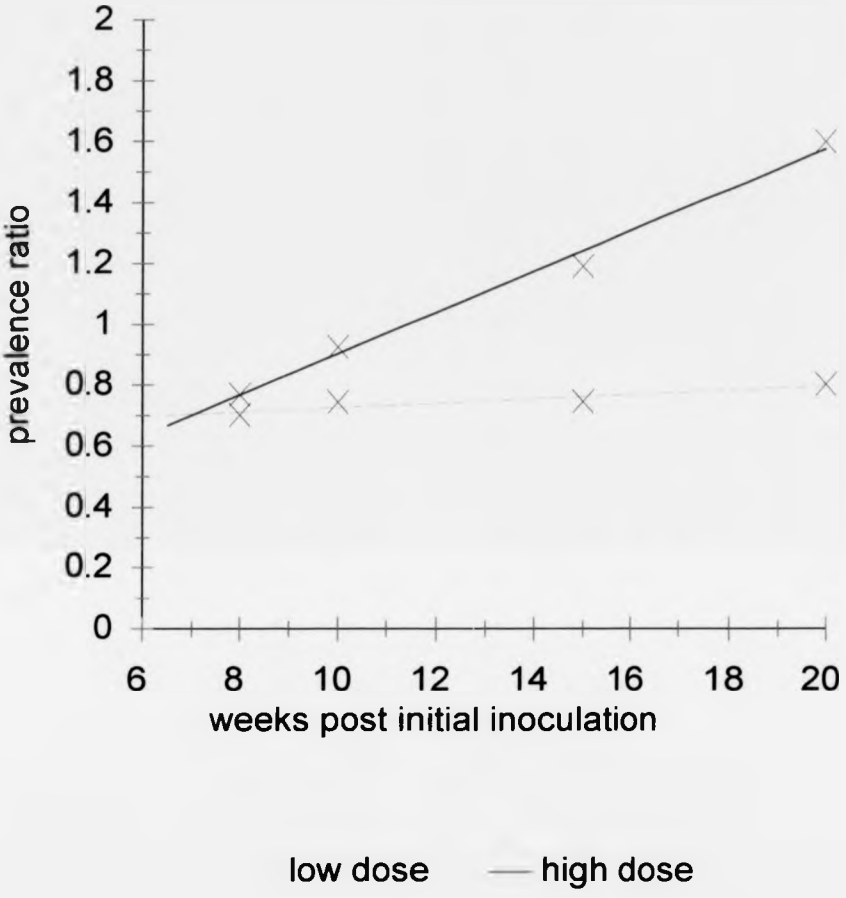


Figure 5.3 The ratio between the observed trickle inoculation coproprevalence from the experiment described in Chapter 4 and the expected single dose prevalence, for a low dose of 100 eggs and a high dose of 6000 eggs per animal. The expected single dose prevalence was obtained using equation 5.5, derived from the relationship between prevalence and dose level following a single experimental inoculation (Roepstorff *et al.* 1997). The x-axis gives time as weeks post initial inoculation for the trickle inoculation coproprevalence data. The trend lines were fitted using regression analysis.

As the expected single dose prevalence remains constant through time for each dose level, figure 5.3 clearly demonstrates that the high dose group in the trickle inoculation

has a higher rate of increase in coproprevalence through time than the low dose group. Note that at week seven the ratio between observed and expected prevalence is the same for both dose groups (approx. 0.7), which is also when the infections are expected to become patent. This is intuitive as the initial coproprevalence observed in the trickle experiment must result from the first inoculation dose given. It is therefore not surprising that it reflects the response to dose observed in a single inoculation. The slight discrepancy between the expected and observed prevalence at this point in time could be due to a number of factors. The most obvious being that the trickle inoculation prevalence is actually the coproprevalence and does not include single sex and single worm infections, which would have contributed to the higher prevalence predicted by the single inoculation worm burden data. Other factors that may contribute to the difference between the observed and expected prevalence at week seven are:

- Differences in egg batches used in the two experiments
- Differences in the age and condition of hosts in the two experiments
- An immune suppression due to the further intake of eggs during the migration and maturation of the worms in the initial dose given to the trickle inoculated animals.

The initial coproprevalence observed in the trickle inoculation was lower than the expected prevalence given by equation 5.5 (see figure 5.3). As there is no way of distinguishing between the possible factors causing this difference, a parameter, c, is introduced to describe this difference. Because of the model assumptions, the initial

coproprevalence can be used directly to represent the initial proportion of "infected" animals at time zero:

$$I_0 = c\left(95 - 18\log_{10}(D)\right) \qquad \textbf{(5.6)}$$

If the difference represented by c was due to the fact that one prevalence resulted from a trickle inoculation and one from a single dose inoculation, c might be the same for all trickle inoculated populations. However, if the difference was due in part to differences in the quality of the egg batch used, or host age and fitness, c would be expected to differ among experiments.

5.3 FITTING THE MODEL TO THE DATA

The model was fitted to the experimental data obtained from the trickle inoculation experiment using the Marquardt method of optimization, see figure 5.4. As in the previous chapter, false positive results were excluded by only counting individuals with a faecal egg count greater than 200 eggs per gram faeces as a positive result.

The parameters calculated by the model were as follows:

$\alpha_1 = 3.1 \times 10^{-6}$

$\alpha_2 = 0.0092$

$c = 0.7$

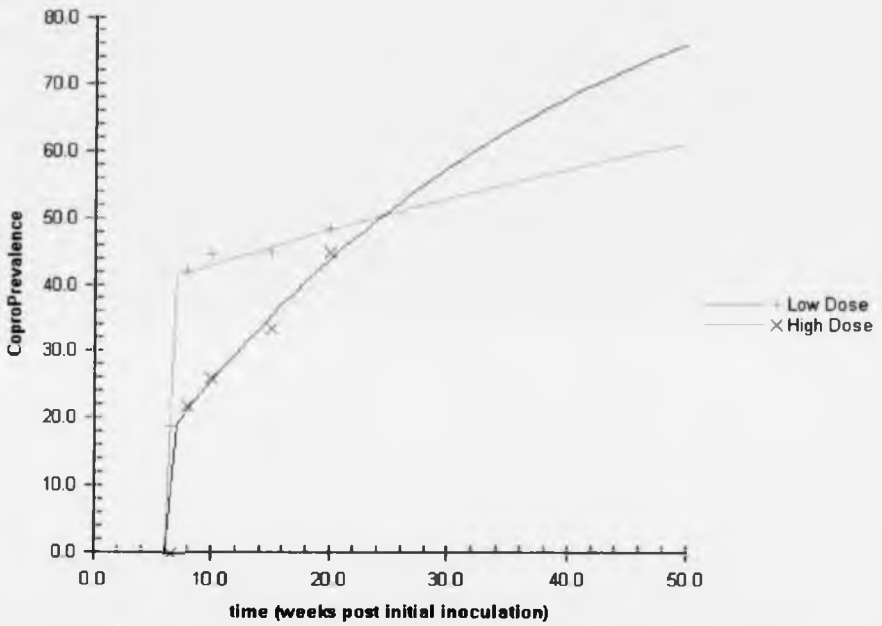


Figure 5.4 The change coproprevalence through time. The Marquardt optimization method was used to fit the model (shown in figure 5.1) to the coproprevalence data from a trickle inoculation experiment described in Chapter 4. Parameters α_1, α_2 and c were estimated. The low dose group was given 100 eggs per animal twice weekly, the high dose group was given 6000 eggs per animal twice weekly. Up to week 10 post initial inoculation, there were 58 animals in the low dose group and 57 animals in the high dose group. After week 10, 29 animals were removed from each group.

5.4 TESTING THE MODEL

Having estimated the parameters, data from two experimental trickle inoculations, as shown in figures 5.5 and 5.6, were used to test the model. The dose was set according to the experimental protocol and all other parameters remained as optimised by the initial data from the trickle inoculation experiment described in Chapter 4.

Data in figure 5.5 is taken from a 12 week trickle inoculation of 38 animals given a dose of 10,000 eggs twice weekly (Boes *et al.* 1998). As a number of pigs were not sampled on both sampling dates, the maximum and minimum prevalences were calculated, such that all the unsampled animals were assumed to be either positive or negative respectively.

Data in figure 5.6 is from a 12 week trickle inoculation of 12 animals given 500 infective eggs twice weekly (Helwigh *et al.* 1999). The 95% confidence intervals are shown.

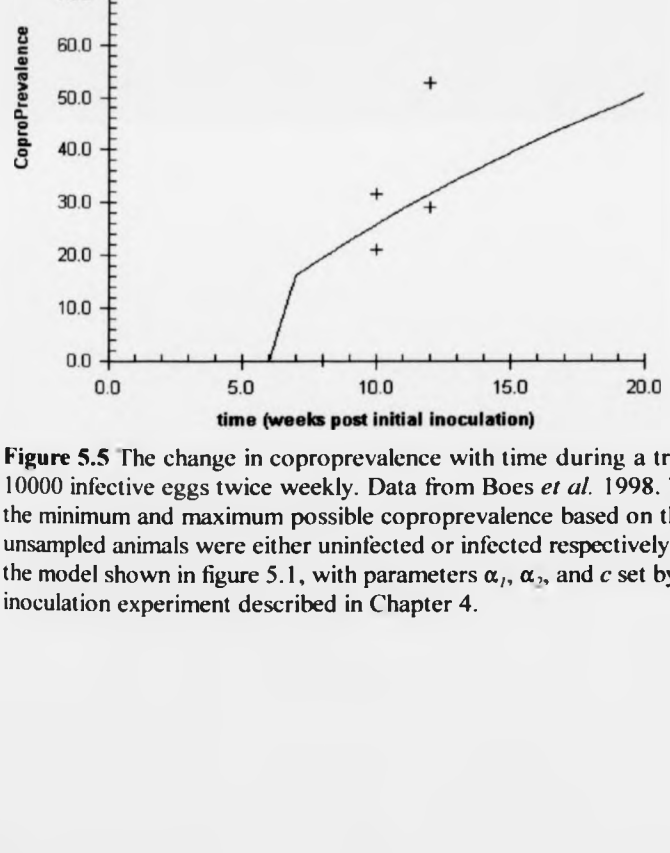


Figure 5.5 The change in coproprevalence with time during a trickle inoculation with 10000 infective eggs twice weekly. Data from Boes *et al.* 1998. The crosses represent the minimum and maximum possible coproprevalence based on the assumption that all unsampled animals were either uninfected or infected respectively. The line comes from the model shown in figure 5.1, with parameters α_1, α_2, and c set by data from the trickle inoculation experiment described in Chapter 4.

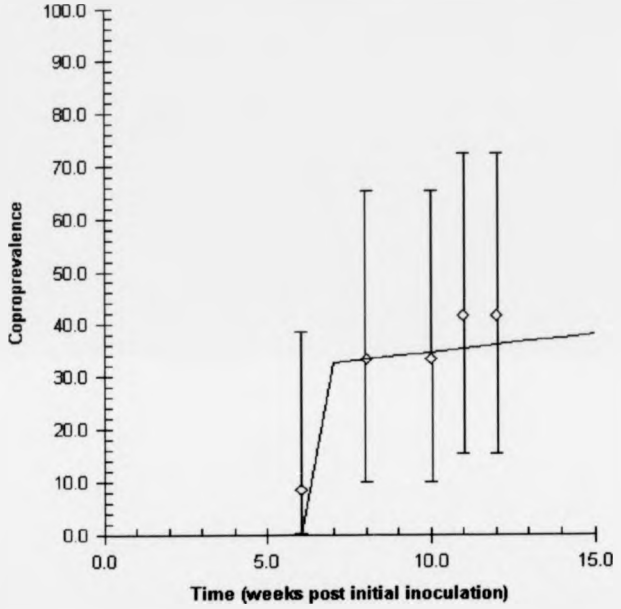


Figure 5.6 The predicted change in coproprevalence (line) with time during a trickle inoculation with 500 infective eggs twice weekly (Helwigh *et al.* 1999), shown as the mean and 95% confidence intervals.

It appears from these figures that the parameters estimated for the model structure by the original data are validated with data from other twice weekly trickle inoculation experiments. This suggests that the difference observed between the initial prevalence following a single inoculation and a trickle inoculation is mainly due to the difference in worm burden prevalence and coproprevalence and the immune response resulting from repeated exposure. Either the differences in egg batches and / or host age and condition were not great in these experiments or they did not have a large influence on the outcome. It also suggests that setting α as a linear function of dose is an acceptable solution, and the assumptions that were made were reasonable.

5.5 THE EFFECT OF DOSE ON COPROPREVALENCE THROUGH TIME

One of the interesting features of the model is the comparable differences in coproprevalence between doses through time. Figure 5.7 shows the predicted coproprevalence up to 40 weeks post initial inoculation (p.i.i.) for three dose levels: 100, 6000 and 10000 infective eggs per animal twice weekly.

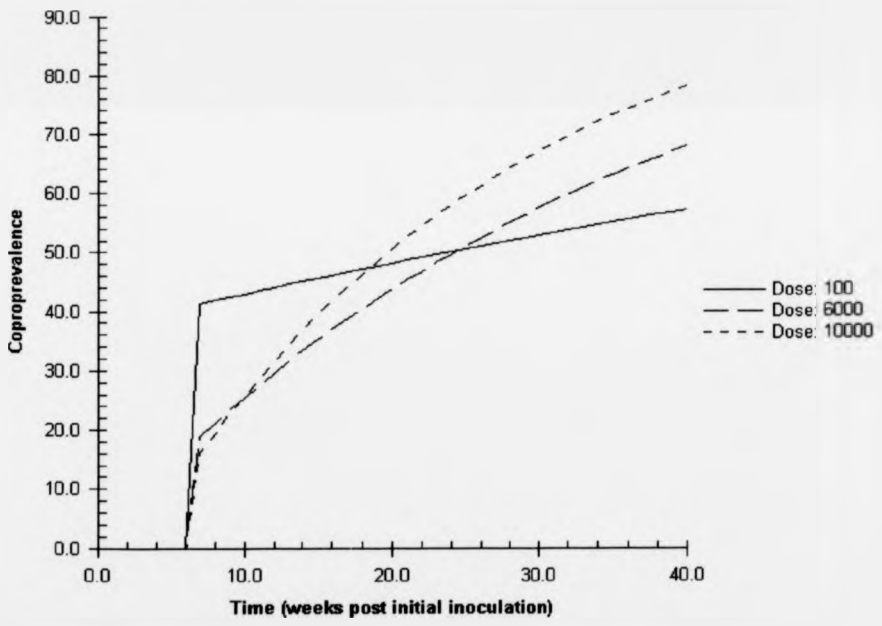


Figure 5.7 Predicted changes in coproprevalence through time at three dose levels (100, 6000 and 10000 eggs per animal twice weekly).

It is striking that a comparison at one point in time of the coproprevalence across the three groups could be very misleading, especially if conclusions on the relative forces of infection were made. At week 10 p.i.i., the intermediate and high trickle inoculation groups have approximately the same coproprevalence, while the coproprevalence of the low dose group is much higher. At week 18 p.i.i., the coproprevalence of the high and low dose groups are equivalent, both being higher than the intermediate dose group. Six weeks later, it is the coproprevalence of the low and intermediate dose groups that are similar. This demonstrates that the history of infection and exposure should be considered before conclusions are drawn when comparing different host populations.

5.6 EXPANDING THE MODEL

Under epidemic conditions is it reasonable to assume that the changing number of infectious individuals would affect the rate of infection and add more complex dynamics to the model. However, under endemic conditions, although seasonal fluctuations are likely to exist, the rate of infection may be stable in the long term. It may therefore be possible to apply the model to age-prevalence data from human communities with stable endemic infections with *Ascaris lumbricoides*, where age is substituted for time and exposure is assumed to start from birth and be constant throughout life.

The data used in this analysis came from a survey by Hall *et al.* (1999) of *A. lumbricoides* infections in Bangladesh where worm expulsion, following anthelmintic treatment, was recorded for 1765 people of all ages. The age-prevalence data for males

in this community was compared to different model outputs. The prevalence in each age class was calculated from the number of people that expelled worms, not the presence of eggs in the faeces. Thus the prevalence in the model is now the true prevalence, and *I* represents true infection. For the purpose of comparison, figures 5.8 and 5. 9 show the two most simple assumptions for a range of doses. First, figure 5.8 demonstrates the assumption that there is no loss of infection and second, figure 5.9 demonstrates the situation where there are no concomitant infections or reinfection. The estimated life expectancy of *A. lumbricoides* is taken as 2 years. To include loss of infection, the model equations were altered as follows:

$$\frac{dI}{dt} = \beta - \nu \tag{5.7}$$

$$\nu = \mu I \tag{5.8}$$

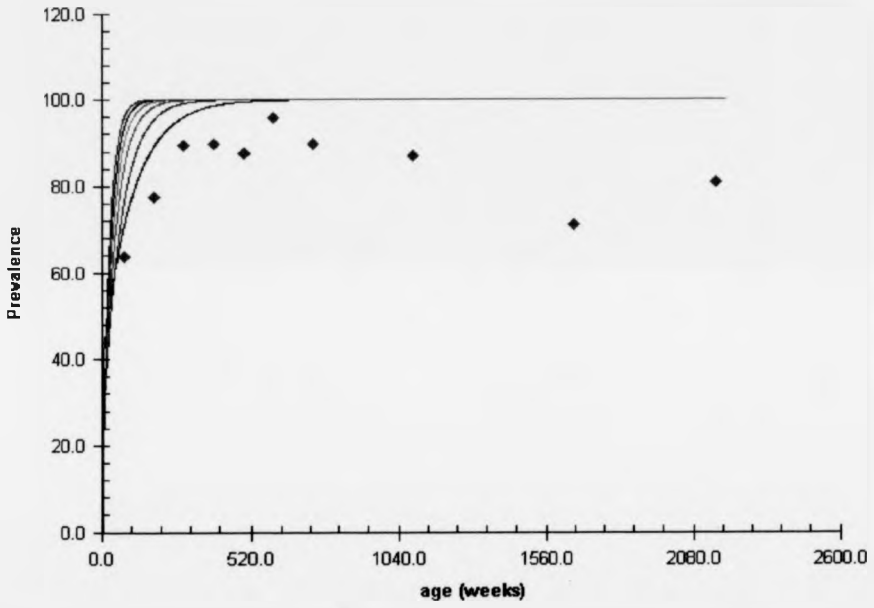


Figure 5.8 The prevalence of infection with *A. lumbricoides* in men in Bangladesh against age, from Hall *et al.* (1999). Model predictions for a range of doses from 100 to 10000 eggs twice weekly where there is no loss of infection.

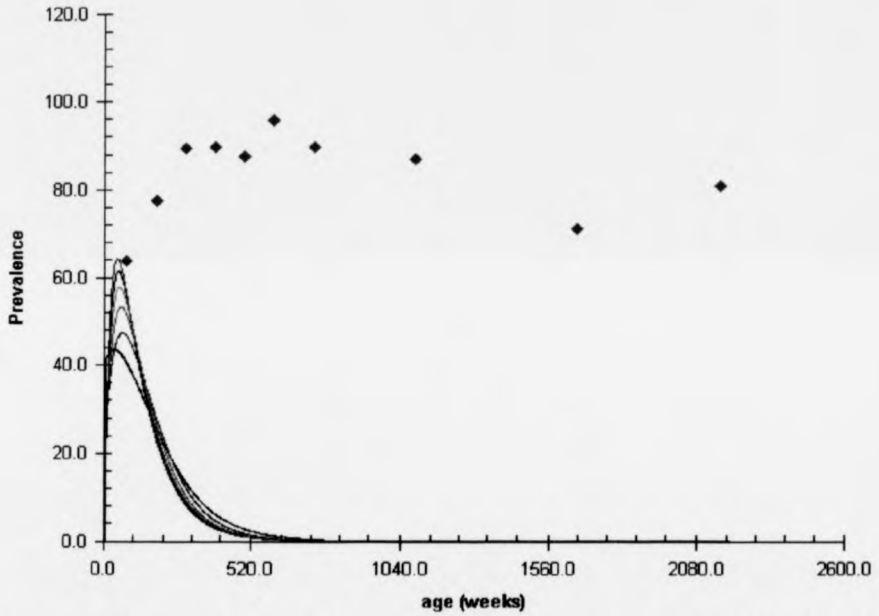


Figure 5.9 The prevalence of infection with *A. lumbricoides* in men in Bangladesh against age, from Hall *et al.* (1999). Dose varies from 100 to 10000 eggs twice weekly, duration of infection 2 years, no reinfection.

It is clear from figures 5.8 and 5.9 that both of these assumptions are too simplistic. Figure 5.8 demonstrates that there must be loss of infection. Figure 5.9 demonstrates that either there are concomitant infections, reinfection following loss or, most likely, both.

In a final analysis, the best fit of the model to coproprevalence data was obtained by optimising the model parameters. To keep the model as simple as possible, it was assumed that concomitant infections occurred, but there was no reinfection. The coproprevalence of each age group was estimated by subtracting 8.2% from the true prevalence. 8.2% was the average difference between coproprevalence and true prevalence given by Hall *et al.* (1999). All the parameters were allowed to vary as it is likely that even though the dynamics of *A. lumbricoides* infections in man may be similar to *A. suum* infections in pigs, the parameters will differ. Although the force of infection cannot be controlled in human infections to obtain values of the model parameters as it was in the pig infections, ideally data from several studies could be compared such that the force of infection was allowed to vary for each site, whilst parameters α_1, α_2 and c were optimised across all data sets.

Figure 5.10 shows the best fit obtained when the model parameters were optimised for the first eight data points. It was apparent that the model did not represent the last data point, which was higher than would be predicted by the model. There are a number of reasons why this could have happened. It is possible that there is a period of immunity after loss of infection, following which reinfection occurs, leading to an increase in the prevalence of the older age classes. There could also be changes in either host behaviour or immunity. Also, the older age classes had a lower mean intensity of infection,

suggesting a greater proportion of single worm infections. In using the average difference in coproprevalence and true prevalence, the coproprevalence could have been overestimated in the older age classes, and underestimated in the younger age classes.

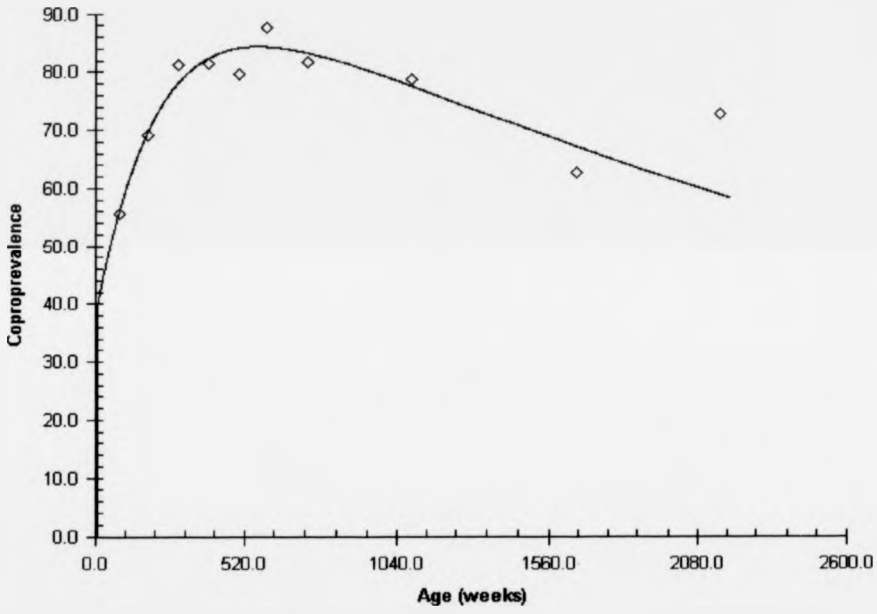


Figure 5.10 Optimising the parameters to fit the model to the estimated coproprevalence from men infected with *A. lumbricoides* in Bangladesh. Data estimated from Hall *et al.* (1999)

The optimised parameters are shown in table 5.1.

Parameter	*A. lumbricoides*	*A. suum*
α_1	3.1×10^{-6}	3.1×10^{-6}
α_2	0.004	0.0092
c	0.71	0.7
D	155	
μ	0.00026	

Table 5.1 The Marquardt optimised parameter values for the *A. lumbricoides* data. The optimised values of a_1, a_2 and c for *A. suum* are shown for comparison.

The rate of increase is slightly lower than it was in the trickle inoculated *A. suum* infections in pigs, though it is still positively associated with dose. The parameters should be estimated for a range of samples to confirm this. There was also no noticeable difference in the parameter c. The force of infection was equivalent to receiving 155 infective eggs twice weekly. The duration of concomitant infections is predicted from μ to be 73 years, suggesting that individuals in this community will be infected throughout life.

5.7 ANALYSIS OF NATURAL INFECTION OF PIGS WITH *A. SUUM*

The model was used to predict the force of infection using data from the natural infection / reinfection experiment by Boes *et al.* (1998). The coprоprevalence was taken at week 8 and 10 post initial exposure. At this stage of infection, any eggs excreted by infected animals would not yet be infectious. Data following treatment was not used as this requires greater complexity than the model is designed for.

The model predicted that level of infectivity on the pasture at this time was equivalent to a twice weekly trickle inoculation per animal of 9107 infective eggs. This is considerably higher than the high dose group, in the trickle inoculation experiment. To investigate the role that force of infection may have played in the different results observed in the natural infection experiment and the trickle inoculation experiment, the true prevalence at treatment and following reinfection was plotted against force of infection for the three groups, see figure 5.11.

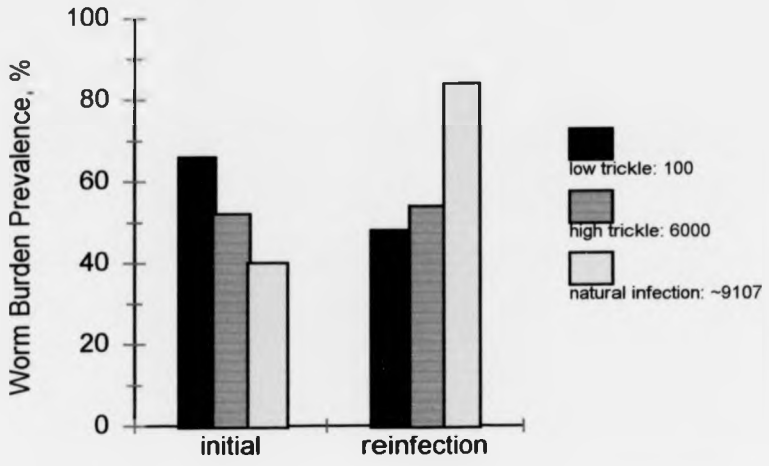


Figure 5.11 The effect of increasing the force of infection on the worm burden prevalence at treatment and following reinfection. Data from the trickle inoculation experiment (Chapter 4) and the natural infection experiment (Boes *et al.* 1998)

In the low dose trickle inoculation group, the prevalence decreased on reinfection. In the high dose trickle inoculation group the prevalence remained approximately the same. In the natural exposure group with the highest estimated force of infection, the prevalence increased on reinfection. A similar trend was observed in the changes in the negative binomial parameter, k. In the low trickle group, k decreased on reinfection, in the high trickle group k was the same at treatment and on reinfection, whilst in the natural infection group k increased on reinfection. From this it can be concluded that the complex relationship between the changes in the worm burden and the force of infection resulted in a decrease in aggregation being observed in the natural infection experiment but not in the trickle inoculation experiment.

CHAPTER 6: DISCUSSION

This chapter aims to summarize the main conclusions, draw together the results of the previous chapters and discuss them in relation to relevant literature. The initial focus will be on the worm burden distribution, with particular reference to the interaction between the aggregation of the distribution and host exposure. The relationship between aggregation and the mean parasite burden will then be considered. Following this, the importance of a host predisposition to light or heavy infection will be examined. The problems arising from the use of coproprevalence as an indirect measure of infection status will then be discussed. The final section will address the population dynamics of *A. suum*. Future perspectives will be highlighted where appropriate. The overall outcome of this research was the use of data available from DCEP to examine factors, such as inoculation protocol and maternal exposure, that influence the population dynamics of *A. suum* infections; the design and execution of an experiment to test the hypothesis that experience of infection lowers the degree of aggregation of parasites among hosts; and the development of a model to explore the relationship between force of infection and prevalence.

In Chapter 2, a linear relationship between the aggregation parameter and the mean intensity of infection was found using data from a series of experiments investigating the effect of maternal exposure on the infection of offspring with *A. suum*. In addition, maximum likelihood was used to demonstrate that the colostrum of previously infected mothers caused the distribution of worms among piglets to become less aggregated. The degree of which was determined by the length of exposure the mother had experienced.

A hypothesis was developed that exposure to *A. suum* may cause a reduction in the aggregation of the worm burden distribution. This was supported by evidence from a natural infection / reinfection experiment (Boes *et al.* 1998) in which the worm burden distribution was observed to be less aggregated following anthelmintic treatment. Further support came from a retrospective analysis of DCEP data, presented in Chapter 3, in which it was shown that worm burdens resulting from trickle inoculations or natural exposure were less aggregated than those resulting from a single inoculation.

The experiment described in Chapter 4 was designed to test this hypothesis by replicating the results of the natural infection / reinfection experiment under more controlled conditions, but produced contradictory results. However in Chapter 5 the new experimental data was used to generate a dynamic model linking prevalence, force of infection and time. This model provided support for a new hypothesis that the difference between the natural infection / reinfection results and those from the trickle inoculation experiment could be explained by differences in the force of infection.

6.1 THE WORM BURDEN DISTRIBUTION

It is often stated that the negative binomial probability distribution provides a good empirical description for the aggregated frequency distributions of parasites among hosts (Anderson & May, 1991; Shaw & Dobson, 1998). This was also found to be true for the distributions of *A. suum* described in this thesis. In all cases, the estimated negative

binomial distributions did not differ significantly from the observed parasite frequency distribution.

Many factors affect the worm burden distribution. Anderson & Gordon (1982) proposed that the resulting distribution may arise from different forces, some of which act to increase over-dispersion and some of which act to decrease over-dispersion. Their theoretical work using Monte Carlo simulations demonstrated that a host mortality rate positively correlated with the worm burden would generate under-dispersion in the worm burden distribution. This is clearly not responsible for the reduction in aggregation observed in the maternal exposure experiments, as host mortality would have been observed in the experimental groups. Nor does it explain the reduction in aggregation when comparing trickle inoculation and natural exposure groups to single inoculation groups. Consequently, the parasite distribution must be determined by the population dynamics of the parasite.

If parasite demographic rate processes were constant one would expect the distribution of parasites among hosts, where there is no direct reproduction within the host, to be governed solely by immigration and death, which would result in a Poisson distribution of parasite burdens between hosts (Bailey, 1964). It is the addition of environmental stochasticity and its effects on the demographic rate processes that generates the typical over-dispersed distribution. Furthermore, it is changes in these processes that must cause aggregation to change. Aggregation in the worm burden distribution has been attributed to the spatial and temporal distribution of infective stages, and differences in host susceptibility, which may be due to behavioural factors, genetic factors or different past

experience of infection (Anderson, 1985; Anderson & Medley, 1985). Although variation in exposure will have a compounding effect on aggregation, the changes in aggregation occur during trickle infections, in which dose is controlled. Likewise, changes in aggregation are seen during experiments, negating the possibility that the change is due to genetic factors (Keymer & Hiorns, 1985). Differences in exposure cannot be used to explain the existence of aggregation. The mechanisms generating parasite aggregation are likely to be multi-factorial, but the main result of this thesis is the demonstration that aggregation may change during exposure in a manner related to the degree of exposure.

Exposure & Aggregation

The analysis in chapter 2 demonstrated the important influence of maternal exposure on the subsequent distribution of worms among offspring. It appears that maternal exposure causes a reduction in the degree of aggregation of the piglets' worm burden distribution, and that the longer the exposure of the sow, the more pronounced the reduction in aggregation becomes. This effect was enhanced by grouping cross-suckled piglets with their suckle mother, rather than their birth mother, suggesting that the colostrum may play an important role in the vertical transmission of immunity against / tolerance to *A. suum* infections. Kelly & Nayak (1965) also proposed that the colostrum would play a significant role in the transfer of maternal factors of importance to *A. suum* infections.

The reduction in aggregation means that at any given intensity of infection, there will be a higher prevalence of infection in the exposed animals. Conversely at a given prevalence the mean intensity of infection will be lower in the exposed animals. Essentially, maternal exposure is likely to result in an increase in the number of offspring with low intensity

infections. The reduction in aggregation may benefit the host, as morbidity is generally associated with high worm burdens (Chan *et al.* 1994).

It is also possible that established parasites may benefit from preventing recruitment of new parasites within the host, especially if there is competition for space or resources.

The retrospective analysis on compiled *A. suum* data from DCEP, demonstrated that not only did trickle inoculations seem to mimic natural infections well, but a significant reduction in aggregation was shown when compared to single dose inoculations. Figure 3.2 clearly shows that the reduction in aggregation of the trickle and natural exposure groups was primarily due to an increase in animals harbouring low-moderate worm burdens. Again suggesting that experience of infection increases the probability of an animal becoming infected at a low level.

The trickle inoculation experiment painted a less clear picture on the relationship between exposure and the degree of aggregation of the worm burden distribution. Duration of infection had no effect on the degree of aggregation, thus although there may be a reduction in aggregation when trickle inoculations are compared to single inoculations, extending the duration of the trickle inoculation does not appear to further reduce the aggregation. This is intuitive, as if the effect of reducing aggregation with experience of infection continued indefinitely, one would eventually observe a Poisson distribution (when k increases to about ten, the negative binomial distribution tends to a Poisson distribution).

The trickle inoculation experiment was originally carried out to test the hypothesis that exposure causes a reduction in the aggregation of the worm burden distribution. It was expected *a priori* that the results would replicate and confirm those found in a natural exposure experiment (Boes *et al.* 1998) in which the distribution on reinfection was observed to be less aggregated that the initial distribution found at treatment. The results however showed no significant difference in the aggregation before and after treatment in either trickle inoculation group, though a slight increase in aggregation was observed in the low dose group. Several hypotheses were put forward (discussed in Chapter 4) to explain the difference in the results between the two experiments. It appears from the modelling results in Chapter 5 that the naturally exposed animals experienced a much greater force of infection than the trickle inoculated animals, and the dynamics of reinfection are dependent upon the force of infection as well as experience. The high force of infection predicted by the model is supported by soil samples taken from the pasture prior to the introduction of the naturally exposed animals. These contained a high concentration of unembryonated eggs (Roepstorff, personal communications), and it is likely that a large number of eggs would have become infective by the time the animals were placed on the pasture in May.

To conclude, the hypothesis that was developed suggesting that exposure causes a reduction in the aggregation of the worm burden distribution was too great a generalisation. There does appear to be a maternal effect in the colostrum, and an initial effect when comparing the increase in exposure of a trickle inoculation or natural infection to a single inoculation. However the reduction in aggregation observed in the natural infection / reinfection experiment (Boes *et al.* 1998) was a symptom of a more

complex dynamic situation. From the model developed in Chapter 5 it appears that these animals were exposed to a much higher force of infection than the animals in the trickle inoculation experiment. Although the "10 weeks infection - anthelmintics - 10 weeks reinfection" inoculation protocol was the same for all three groups, the balance between immunity and unresponsiveness was dependent upon the relationship between time and force of infection. Having demonstrated an effect of maternal exposure on the distribution of worms among piglets, an interesting area of future research may be to investigate the influence of neonatal infection on the distribution of worms among older animals.

Force of Infection & Aggregation

The force of infection in the trickle inoculation experiment seemed to be positively associated with aggregation; the low dose trickle groups had less aggregated worm burdens than high dose trickle groups. Even though this was observed both after 10 weeks trickle inoculation and 20 weeks trickle inoculation, the difference was not great enough to be significant. A Monte Carlo simulation, using the same procedure as in chapter 3, revealed that if there was a real effect of the size of the inoculation dose on the aggregation of the worm burden distribution, given the distributions observed at 10 weeks trickle inoculation, over 100 animals would be needed to guarantee with a power of 95% a significant difference in aggregation at the 95% level. In addition to this being a logistically difficult number of animals, and an expensive experiment to perform, evidence from a separate experimental trickle inoculation indicates that in spite of the results being replicated at 10 and 20 weeks, in two groups of animals, it is not a real effect. Boes *et al.* (1998) performed an experimental trickle inoculation of 38 animals

with 10000 eggs twice weekly, which resulted in a worm burden distribution with an aggregation parameter k of 0.26. The force of infection used was greater than that of the high dose group yet the distribution was much less aggregated than the low dose trickle inoculation group, indicating that the observed trend for aggregation to increase with force of infection, even though it was observed on two separate occasions in different groups of animals, was again an artifact of a more complex dynamic situation. This highlights the problem with this type of epidemiological data, that trends are often drawn from distribution parameters, resulting in a very low sample size, even though the initial distribution may have consisted of large samples.

6.2 THE DEPENDENCE OF THE AGGREGATION PARAMETER, k, ON THE INTENSITY OF INFECTION

In the maternal exposure experiment a linear relationship between the aggregation parameter k, and the mean intensity of infection was found. The intercept was governed by the duration of maternal exposure, while the slope remained common across all groups, indicating a second mechanism independent of the maternal exposure. In a study of the parasite abundance and aggregation in wildlife populations, a strong linear relationship was found between the log variance and log mean of the intensity of infection (Shaw & Dobson, 1995), suggesting that parasites are constrained in the degree of variation for any given mean. Given the common observation of reduced aggregation with increasing mean burden for a range of parasites (Guyatt *et al.* 1990, Lwambo *et al.* 1993, Guyatt *et al.* 1994) a general mechanism may be involved. This could be the result

of an evolutionary trade-off between the parasites becoming too aggregated (all parasites are accumulated in one host) or too random (reduced probability of mating) in their distribution (Anderson & Gordon, 1982).

Another possible explanation is that the reduction in aggregation with increasing mean is simply a result of a maximum attainable worm burden truncating the tail of the distribution. Distributions with high mean intensities, have a greater probability of finding a sample in the tail of the distribution, see figure 6.1. Theoretically the tail of the negative binomial distribution extends to infinity, and it is on this principle that the likelihood is calculated. Preventing this tail from extending past a given point would act to reduce the aggregation (increase k), albeit with a small reduction in the mean. The reduction in aggregation caused by the truncation of the tail would be more pronounced at higher mean worm burdens, and thus would be correlated with worm burden.

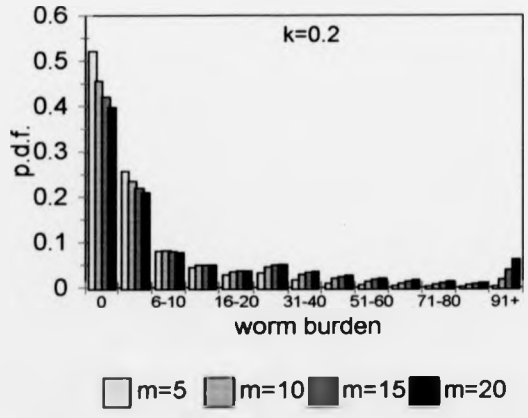


Figure 6.1 The probability density function for the negative binomial distribution with a mean ranging from 5 to 20, and aggregation parameter k =0.2, demonstrating the higher probability of obtaining a sample in the tail of the distribution with increased mean intensity.

A maximum attainable worm burden could be a product of competition for space or resources (Shaw & Dobson, 1995). This may be particularly likely if, as in this case, the hosts were very young. If the truncation of the tail of the distribution was the cause of the linear relationship between the aggregation parameter *k* and the mean worm burden, an asymptotic relationship between the maximum worm burden and the mean worm burden across distributions would be expected. To demonstrate that this was not the case, the maximum worm burden was plotted against the mean worm burden for the distribution of worms in piglets grouped by their suckle mother in figure 6.2(a), and the worm burden distributions of the older hosts used in the investigation of inoculation protocol (chapter 3) and the trickle inoculation experiment (chapter 4), in figure 6.3(b).

It is clear from these figures, that although there is a reduction in the maximum worm burden in the piglets compared to the adult pigs, this occurs at all mean worm burdens. More importantly there appears to be a linear relationship between mean and maximum worm burden for all animals, which implies that the linear relationship between the mean intensity of infection and the aggregation parameter *k* was not due to the truncation of the negative binomial distribution. Further support comes from field studies in which worm burdens up to 1800 have been observed (Ajayi & Arabs, 1988).

The reduction in the maximum worm burdens of the piglets when compared to the adult pigs may be due in part to the greater competition for space, however the number of infective eggs given to the piglets will have also been a factor. The piglets were infected with either 2 or 3 doses of 50 eggs (see Boes *et al.* 1998), which provides an upper limit for the number of worms that the piglets could obtain.

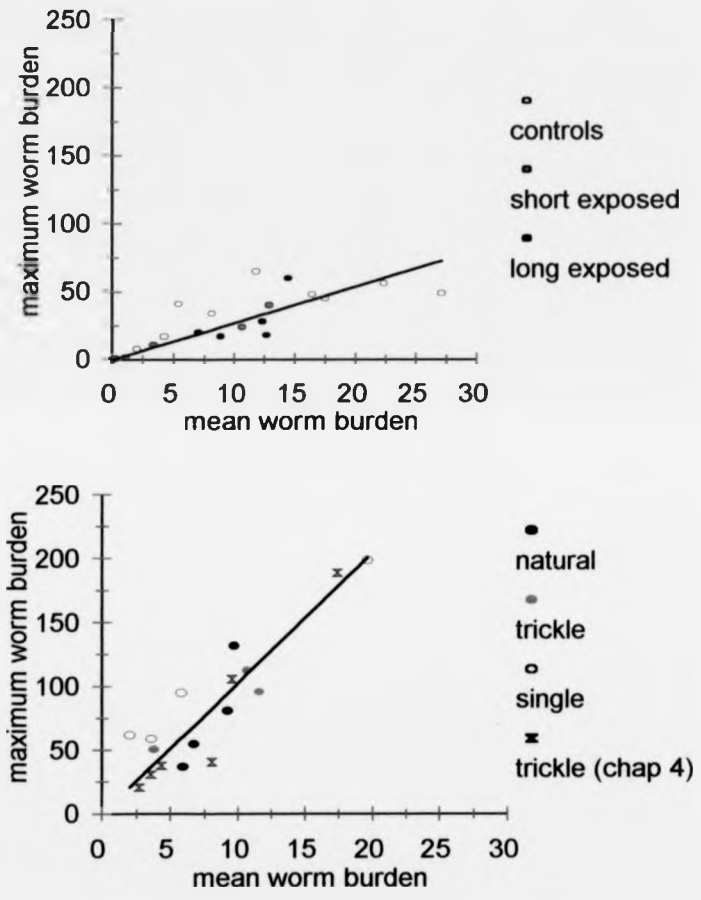


Figure 6.2 The relationship between maximum and mean worm burdens of (a) piglets (data from Chapter 2) and (b)pigs (data from Chapters 3 & 4)

Although an absolute truncation of the tail of the distribution is not responsible for the linear relationship between the mean and the degree of aggregation, it is still possible that density-dependent limitations such as reduced parasite survival or establishment with increased intensity of infection may be the mechanism. This hypothesis is supported by theoretical work by Anderson & Gordon (1982) and Pacala & Dobson (1988).

The linear relationship between k and the mean intensity of infection has been previously described for *A. lumbricoides* infections in humans, where the distribution of worms was compared across communities from a wide range of geological, climatic and environmental locations (Guyatt *et al.* 1990). In addition to the mechanisms already discussed, it was suggested that the association between the mean intensity of infection and the degree of aggregation could be a function of human behaviour, such that an enhanced awareness of infection may encourage self-treatment, particularly in individuals with the most overt clinical symptoms. This may be a factor in human infections, but it was not a factor for the experimental pig infections.

The fact that a linear relationship was observed in both human and pig *Ascaris* infections provides strong evidence for density-dependent limitations on either establishment or parasite survival. Although host mortality and self-treatment may contribute to a reduction in aggregation in natural infections, they are not the sole factors, as they were absent from the experimental infections and a reduction in aggregation with increased mean intensity of infection still occurred.

6.3 PREDISPOSITION

As a result of the highly aggregated nature of the distribution of worms among hosts, the majority of worms are harboured by the minority of hosts (Anderson & Gordon, 1982). A predisposition of certain hosts to heavy infection could have important consequences, both clinically and for the transmission dynamics (Anderson & Medley, 1985). There is considerable evidence for the existence of a predisposition to heavy or light infection in human *A. lumbricoides* infections (Haswell-Elkins *et al.* 1987, Holland *et al.* 1989, Hall *et al.* 1992). Although the generative processes remain unknown, genetic, behavioural and social factors, as well as nutritional status, have all been suggested as possible causes (Anderson, 1989).

The trickle inoculation experiment revealed a strong positive correlation between the intensity of infection found at treatment and at necropsy. As the host nutritional status and the distribution of infective stages were controlled, it is likely that the observed predisposition was due to genetic factors. A genetic epidemiological study of *A. lumbricoides* infections in humans revealed a strong genetic component accounting for between 30% and 50% of the variation in worm burden (Williams-Blangero *et al.* 1999) There is evidence from human studies for a possible genetic factor influencing protection from infection with *A. lumbricoides* (Holland *et al.* 1992). A similar mechanism may be responsible for the predisposition observed in the present study as the correlation was strongly influenced by animals remaining uninfected on both occasions. There is also tentative evidence from human studies for a genetic basis of enhanced susceptibility to intense infection (Bundy, 1988). Again, a similar mechanism may have contributed to the

predisposition observed in the present study, as the most heavily infected animals found at necropsy (those in the "tail" of the distribution) were also heavily infected at the time of treatment. Future experiments examining serological correlates under conditions of controlled exposure may provide further insight into the processes generating the observed predisposition.

The correlation coefficients found in the present study were remarkably similar to those found in the natural exposure experiment (Boes *et al.* 1998). This indicates that the predisposition observed in the naturally infected animals may have also been primarily caused by genetic factors, suggesting that environmental and behavioural factors did not have a large influence (nutritional status was again controlled). In addition, the correlations found in both of these experiments were comparable to those observed in human studies, providing further support for the importance of genetic components to predisposition. However, the difference in the duration of exposure to infective stages between the human studies and the pigs studies should be borne in mind. In the pig experiments the duration of both the initial infection and the reinfection phase was 10 weeks. In the human studies, reinfection periods ranged from 6 months to 17 months. It has been suggested that increasing the period between treatment and reinfection would act to increase the level of correlation observed (Keymer & Pagel, 1990).

In the present study, a strong positive correlation was also found between the number of mature worms and the number of immature worms obtained at necropsy. Despite being exposed to infective stages, some animals were found to have no immature or mature worms, indicating that following long term exposure, these animals became

resistant to infection, prior to the self-cure mechanism. The correlation also demonstrates that the presence of a patent infection does not appear to prevent new worms from migrating.

Certain hosts appear to be predisposed to heavy infection or protected from infection despite exposure to infective stages. The results presented in the present study indicate that genetic factors play an important role. Nutritional factors may compound the situation but predisposition is observed in both well nourished pigs and malnourished humans. A comparison of the correlations between the present study and the natural exposure experiment suggests that behavioural and environmental factors do not have an important influence on predisposition in pigs. Further experiments need to be done to elucidate the mechanisms generating predisposition, with particular attention paid to antigenic markers for protection or enhanced susceptibility. It appears from this study that the pig is a suitable model to study genetic components of predisposition in human infections.

A greater understanding of the processes that may cause the predisposition of certain individuals to heavy infection (and thus to disease) is of considerable relevance to the design of control programmes (Keymer & Pagel, 1990). However, selective treatment for heavily predisposed individuals is no longer considered a viable strategy due to the high proportion of heavily infected children in communities with high prevalence, poor compliance and the lack of simple cost-effective methods for identifying 'wormy people' (Albonico *et al.* 1998). Universal treatment and treatment targeted at children have been

shown to significantly reduce the mean intensity of infection within a community (Asaolu *et al.* 1992) and are, at present, considered to be more suitable control strategies.

6.4 THE USE OF FAECAL EXAMINATION TO DETERMINE PREVALENCE

In the model described in chapter 5, coproprevalence was used to reflect the change in the number of infected animals through time. In this section, the relationship between coproprevalence and true prevalence is discussed, to understand how useful a model based on coproprevalence rather than true prevalence would be, and ascertain what conclusions about the true level of infection can be drawn from such a model.

Coproprevalence is defined as the proportion of the host population that have had *A. suum* eggs positively identified in a faecal sample. Essentially, faecal examination provides a surrogate test for identifying individuals that are infected with *A. suum*. The main advantages of using coproprevalence are that infection status can be monitored without altering the infection, which is important under experimental conditions, and that it is easy to perform (Shaw & Dobson, 1995). The main disadvantage of using coproprevalence is that it does not give a true picture of the infection status of a population.

Coproprevalence can be erroneous in two ways. Firstly, it has a low sensitivity which acts to under-estimate the true prevalence. This is primarily caused by unisex and immature infections which remain undetected by faecal examination. Secondly, and

conversely, it has a low specificity which acts to over-estimate the true prevalence. This occurs when eggs are ingested and subsequently detected in the faeces but the host is not infected (Boes *et al.* 1997). It is the balance between these two factors that determines whether the coproprevalence will over-estimate or under-estimate the true prevalence.

Table 6.1 shows the true prevalence, coproprevalence, the proportion of false negative and false positive samples, and the sensitivity and specificity of the faecal examination, at necropsy for the trickle inoculation experiment described in Chapter 4. Sensitivity is defined as the ability of a test to correctly identify an infected individual while specificity is the ability of a test to correctly identify an uninfected individual (Martin *et al.* 1987). It can be seen from the overall sensitivity and specificity that in this case the coproprevalence underestimated the true prevalence. Overall the true prevalence of infection at necropsy was 60.9%. Only 42.6% of animals were positively identified by faecal examination.

Table 6.1 A comparison of true prevalence and coproprevalence at necropsy for the animals in the trickle inoculation experiment

	Group				Overall
	1B	2	3B	4	
Number of animals	29 (100%)	29 (100%)	28 (100%)	29 (100%)	115 (100%)
Number infected	14	21	15	20	70
True prevalence	48.3%	72.4%	53.6%	69.0%	60.9%
Eggs detected	8	17	7	17	49
Coproprevalence	27.6%	58.6%	25.0%	58.6%	42.6%
False negative	6	5	9	5	25
	20.7%	17.2%	32.1%	17.2%	21.7%
False negative with	5	3	6	5	19
unisex worm burdens	17.2%	10.3%	21.4%	17.2%	16.5%
False positive	0	1	1	2	4
	0.0%	3.5%	3.6%	6.9%	3.5%
False positive	0	1	0	2	3
with egp<200	0.0%	3.5%	0.0%	6.9%	2.6%
False positive, likely	0	0	1	0	1
expulsion of worms	0.0%	0.0%	3.6%	0.0%	0.9%
Sensitivity	57.1%	76.2%	40.0%	75.0%	64.3%
Specificity	100%	87.5%	92.3%	77.8%	91.1%

The overall specificity of 91.1% implies that in this experiment the number of false-positive samples was low. The experimental design was chosen to limit as far as possible any additional ingestion of infective eggs from the environment. To achieve this, the animals were moved to a clean pasture every three weeks, once egg excretion had been detected. It appears that this design also reduced the number of false-positive samples that were recorded. For comparison, in a study on false-positive *A. suum* egg counts in

pigs, Boes *et al.* (1997) cited a level of 14% false positive samples for pigs kept on pasture. In the present study there was an overall level of 3.5% false-positive identification.

Roepstorff & Nansen (1998) suggested that false-positive identification could be minimised by excluding animals with an EPG less than 200, as a single adult female would normally produce 400 - 800 eggs per gram of faeces. Further inspection of the data shows that false-positive samples attributed to geophagia and / or coprophagia only occurred in animals in the untreated groups. One false-positive animal was identified in group 3B (high dose, treated) but examination of the EPG history suggests that the worms were expelled shortly before necropsy. The most likely reason for finding more false-positive samples in the untreated groups, is that there was a significantly higher true prevalence in these groups than in the treated groups. As the pasture was clean when the animals were moved there, environmental contamination has occurred as a result of infected animals in the group. Not only was the prevalence of infection higher in the untreated groups, but the worm burdens that they harboured were older, and as a consequence more fecund.

It is apparent that for these data, the discrepancy between true prevalence and coproprevalence is primarily due to false-negative samples. Guyatt & Bundy (1993) proposed a model for predicting the level of false-negative samples due to single worm or single sex infections that would be expected for parasites with an aggregated distribution that could be described using the negative binomial distribution. Unfortunately this model can not be applied to the dynamic model of coproprevalence

to give an indication of the true prevalence, as it relies on a knowledge of the mean intensity and aggregation of infection. However it can be used to explore the factors that affect the extent to which false-negative samples will under-estimate the true prevalence. For example, it was shown that the less aggregated the distribution was, the more the true prevalence would be underestimated by faecal examination. Equally, one could show that the lower the mean intensity of infection became, the more the true prevalence of infection would be underestimated by faecal examination.

One interesting feature of the model by Guyatt & Bundy (1993) is that it allows for different assumptions to be made about the sex ratio and whether or not single female worms will continue to produce eggs in absence of a male. When the model was applied to the data for the following four scenarios:

(i) Sex ratio is unity; females produce eggs in absence of males,

(ii) Sex ratio is unity; females do not produce eggs in absence of males,

(iii) Sex ratio is taken from data; females produce eggs in absence of males,

(iv) Sex ratio is taken from data; females do not produce eggs in absence of males,

the proportion of false negative samples predicted by the model were 7.7%, 15.4%, 5.2% and 16.3% respectively. As is predicted in the paper, the level of false-negative samples is much higher when female only infections remain undetected. It was also predicted in the paper, that divergence from a 1:1 sex ratio would increase the number of false-negative samples when females were undetected, but would decrease the number of false-negative samples as the worm burden became female biassed if females were detected. For these data, as is typical for *A. suum* and *A. lumbricoides* infections, the worm burdens were female biassed.

A comparison of the model predictions to the real data shows that the assumptions given in statement (iv) best represent the observed situation, where false-negative samples that were attributed to unisex worm burdens accounted for 16.5% of the host population. It appears that the most important factor in predicting the level of false-negative samples, is the assumption that females will not produce eggs in absence of males. Inspection of the raw data reveals that female worm burdens were only associated with an EPG of zero or twenty. Incorporating the true sex ratio increases the accuracy given this statement, but decreases it if it is assumed that female only infections will be detected. The conclusion that female only infections will remain undetected by faecal examination supports experimental evidence by Jungersen *et al.* (1997), which demonstrated that transplanted female worms continued to produce eggs for just a limited period of time when isolated from males.

Inspection of the data shows that although 16.5% of the population were infected but remained undetected due to unisex infections, 21.7% in total of the population were false-negative. The additional 5.2% is likely to be due to immature infections or unmated pairs of worms, though the fluctuations in egg production by the worms and faecal production by the host as well as experimental accuracy may be responsible.

When applying the dynamic model of coproprevalence, described in chapter 5, the contribution of false-positive samples was minimised by following the rule suggested by Roepstorff & Nansen (1998) that an EPG of less than 200 was likely to be a false-positive result. This was done so that data from different experiments would have a greater commensurability with each other, regardless of the management of the hosts.

The type of management can strongly influence the occurrence of false positive samples, Boes *et al.* (1997) reported that pigs housed indoors had 28% false-positive samples whilst pigs housed outdoors had only 14% false-positive samples. The present investigation has also shown that a considerable reduction in the number of false positive samples recorded occurs in pigs that have recently been moved to a clean environment.

The most influential factor in determining the level of false negative results appears to be the parasite biology. It has been suggested that *A. suum* female worms are only capable of producing eggs for a limited period of time after isolation from males (Jungersen *et al.* 1997), thus most female infections will remain undetected by faecal examination. Conversely, it has been suggested that female *A. lumbricoides* will produce eggs in absence of males (Guyatt & Bundy, 1993). It would be surprising for two such closely related species to differ in this way, suggesting that further work needs to be done to elucidate the situation. One difficulty is that in studies of human infection with *A. lumbricoides*, anthelmintic treatment is often only given to individuals with positive faecal samples (Thein-Hlaing *et al.* 1984; Bundy *et al.* 1987). This is in part due to the labour involved in determining worm burden through anthelmintic expulsion but there may also be ethical reasons for withholding drugs from individuals, especially children, with negative faecal samples. It is possible that the difference between the detection of female only infections in *A. suum* and *A. lumbricoides* is not due to the biology of the parasite, but rather the method of detection used. In *A. suum* studies coprological surveillance is normally done using the McMaster technique (Roepstorff & Jorsal, 1989; Roepstorff, 1997; Roepstorff *et al.* 1998), whilst *A. lumbricoides* studies typically use

the Kato-Katz method (Asaolu *et al.* 1992; Holland *et al.* 1996; Peng *et al.* 1998b) which may be more likely to detect unfertilized eggs.

The dynamic relationship between the mean intensity of infection, the aggregation and the prevalence adds to the difficulty in interpreting coproprevalence and comparing coproprevalence across different groups. The retrospective analysis of data in Chapter 3, indicated that when progressing from a single inoculation to repeated inoculations, the mean would increase and the aggregation would reduce. The model by Guyatt & Bundy (1993) revealed that an increase in the mean would result in a reduction of false-negative samples, however, to counteract this, the reduction in aggregation would increase the probability of finding a false-negative sample. Data from the trickle inoculation experiment did not demonstrate any significant difference in the mean or the degree of aggregation when comparing the worm burden distributions after 10 weeks trickle inoculation with those from 20 weeks trickle inoculation, though the mean intensity of infection at 20 weeks in the high dose group was considerably larger than at 10 weeks. This suggests that in the high dose group, the level of false negative samples may have reduced with time, emphasising the increase in coproprevalence through time. In conclusion, the power of coproprevalence to reflect true prevalence may not be constant through time and will be affected by the change in the distribution, thus some care needs to be taken when interpreting the results of the model described in Chapter 5.

6.5 MODELLING THE POPULATION DYNAMICS OF *ASCARIS*

Dietz (1982) concluded that due to dynamics of helminth infections, prevalence models would be unsuitable, as two communities may exhibit the same prevalence profile yet experience different levels of infection intensity. Although the prevalence model developed in Chapter 5 took a very basic approach, it produced some interesting and important results about the dynamic relationship between prevalence and force of infection. Firstly, it clearly demonstrated that comparisons of prevalence across different host populations through time could be clearly misleading if the history of infection was not taken into consideration. Secondly, it showed that although a high force of infection will initially invoke a stronger immune response than a low force of infection, through time persistence and unresponsiveness will develop more quickly. This is in agreement with a model by Anderson (1994) which indicated that high exposure eventually resulted in the persistence of infection whilst low exposure resulted in immunity.

The model developed in Chapter 5 was a new approach to examining the dynamics of helminth infections. The data available were used to model the rate that new infections were acquired as a function of dose. It would be theoretically interesting to further expand the model to include loss of infection and reinfection, though in practical terms this would be difficult to achieve. However, the incorporation of age-prevalence data from endemic communities, such as the data set used from Hall *et al.* (1999), may provide further insight into these dynamics. This would also enable more accurate parameter estimates to be obtained.

The results of this work have brought useful insight into the dynamics of *A. suum* infections, in terms of the prevalence of infection and the aggregation of the distribution. A clear relationship exists between the prevalence and mean intensity of infection at a given point in time. Future work should focus on incorporating the intensity of infection into the dynamics of prevalence and aggregation, as disease is generally associated with high worm burdens.

APPENDIX A: CODE FOR MATLAB® TO SAMPLE FROM THE NEGATIVE BINOMIAL DISTRIBUTION

```
%negbin (k,m) samples the negative binomial distribution with
%aggregation parameter = k and mean = m

function y=wb(k,m)

SUM = 0; w=0; success=0;

U=rand(1,1);      %uniformly distributed random number between 0 and 1

for w = 0:inf  %starting at worm burden w=0

%calculate probability of worm burden w coming from neg bin
%distribution with k and m:

p_smk = ((gamma(k+w))/(gamma(k)*gamma(w+1)))*...
    ((m/(k+m))^w)*...
    ((k/(k+m))^k);


SUM = SUM + p_smk;   %calculates cumulative probability for 0 to w %worms

if SUM >= U              %if SUM is greater than generated random number,
```

APPENDIX A: CODE FOR MATLAB® TO SAMPLE FROM THE NEGATIVE BINOMIAL DISTRIBUTION

```
%negbin (k,m) samples the negative binomial distribution with
%aggregation parameter = k and mean = m

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%distribution with k and m:

p_smk = ((gamma(k+w))/(gamma(k)*gamma(w+1)))*...
   ((m/(k+m))^w)*...
   ((k/(k+m))^k);


SUM = SUM + p_smk;   %calculates cumulative probability for 0 to w %worms

if SUM >= U          %if SUM is greater than generated random number,
```

```
   worms=w;                %worm burden equals current value of w

if SUM >= U
break

end
end
end

y=worms;
```

APPENDIX B: COMPARING TWO SAMPLED DISTRIBUTIONS

```
% input Nsim, the number of simulations to be performed
% input Npig, the number of pigs in each group
% input ka and ma, the neg bin agg parameter and mean of
% distribution a
% input kb and mb, the neg bin agg parameter and mean of
% distribution b

% program samples "Npig" pigs from distributions a and b and
% assesses whether sampled distributions are better described by
% individual parameters or joint parameters

% Repeats Nsim times

% "success" gives list of successes and failures of each trial
% (simulation)
% "sum(success)/Nsim" gives prob of obtaining a success with Npigs
% per group, given distributions.

success=zeros(1,Nsim);  %creates empty matrix for success or failures
                        %to be entered on each simulation
```

```
for n=1:Nsim,  %starting at simulation 1 and repeat until Nsim
                %simulations performed
   A=zeros(1,Npig);  %creates empty matrix for sample distribution A,
                %size Npig
   B=zeros(1,Npig);  %ditto sample distribution B (from neg bin distn
                %b)
   C=zeros(1,(2*Npig));  %ditto sample distribution C

% sample distribution C is a combination of A and B so it's size is
%double Npig
% C is used to compare likelihood of A + likelihood of B, given
%their parameters, with the likelihood of C, given its parameters.

   for i=1:Npig,  %for each pig in the group, starting at 1 and
                %finishing at Npig
      A(i)=negbin(ka,ma);  %calculate a worm burden using program
                %"negbin" based on the negative binomial
                %distribution with k of ka and mean of ma
      B(i)=negbin(kb,mb);  %ditto for neg bin distn with k or kb and
                %mean of mb
   end

   C=[A B];  %C is simply the distributions of worm burdens from both
                %A and B
```

```
m_A=mean(A); %m_A is the mean of the sampled distribution of A
s_A=std(A); %s_A is the standard deviation of the sampled
%distribution A
k_A=(m_A^2/((s_A^2)-m_A));
                %calculated the moment estimate of neg
                 %bin k from mean and standard deviation


m_B=mean(B);
s_B=std(B);
k_B=(m_B^2/((s_B^2)-m_B));

m_C=mean(C);
s_C=std(C);
k_C=(m_C^2/((s_C^2)-m_C));

neglog_A=zeros(1,Npig); %creates empty matrix for the negative
                        %log likelihood for the worm burden of
                        %each pig in group A
neglog_B=zeros(1,Npig);
neglog_C=zeros(1,(2*Npig));

for j=1:Npig, %for pig "1" to pig "Npig" in distribution A and B,
              %calculated negative log likelihood of worm burden
```

```
neglog_A(j)=
   -log((gamma(k_A+A(j))/(gamma(k_A)*gamma(A(j)+1)))*
   ((m_A/(m_A+k_A))^A(j))*((k_A/(k_A+m_A))^k_A));

neglog_B(j)=
   -log((gamma(k_B+B(j))/(gamma(k_B)*gamma(B(j)+1)))*
   ((m_B/(m_B+k_B))^B(j))*((k_B/(k_B+m_B))^k_B));

      end

    for l=1:(2*Npig),  % for pig "1" to pig "2*Npig" in distribution
                       %C, calculate negative log likelihood of worm
                       %burden
neglog_C(l)=
   -log((gamma(k_C+C(l))/(gamma(k_C)*gamma(C(l)+1)))*
   ((m_C/(m_C+k_C))^C(l))*((k_C/(k_C+m_C))^k_C));

        end
           if  sum(neglog_C) -
   (sum(neglog_A)+sum(neglog_B))>2.995
       success(n)=1;  %if the difference in total negative log
                      %likelihood between C and (A+B) is greater than
                      %2.995, then success of simulation is 1
    end
             %repeat for next simulation until all simulations are complete
   end
```

APPENDIX C: RAW DATA FOR THE TRICKLE INOCULATION EXPERIMENT DESCRIBED IN CHAPTER 4

Group I		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph.	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
1	100	0	9	3	0	12	2840	7	5	1	0	0	0	0	0	0	0	0
2	100	0	16	10	15	41	7380	2	0	1	0	2	2	1	0	0	1	0
3	100	0	6	0	2	8	300	1	2	1	0	1	1	0	1	0	1	0
4	100	0	2	0	1	3	0	0	0	1	1	3	4	0	0	0	0	0
5	100	0	14	7	0	21	2940	3	0	1	0	5	5	3	2	0	5	820
6	100	0	0	0	0	0	0	8	0	1	1	0	1	0	0	0	0	0
7	100	0	3	0	0	3	1500	1	0	1	0	0	0	0	0	0	0	0
9	100	0	11	1	3	15	9600	0	0	2	1	1	2	6	1	0	7	1160
10	100	0	5	2	1	8	2740	0	0	1	0	1	1	0	1	0	1	0
11	100	0	18	6	2	26	4220	0	0	2	0	1	1	0	1	0	1	0
12	100	0	0	0	0	0	20	3	2	1	0	0	0	0	0	0	0	0
13	100	0	0	1	0	1	0	0	2	1	0	2	2	0	0	0	0	0
14	100	0	2	2	1	5	240	0	1	1	1	0	0	0	0	0	0	0
15	100	0	0	0	0	0	0	2	12	1	1	1	2	1	0	0	1	0

Group 1 cont.		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph.	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
16	100	0	0	0	0	0	0	0	1	1	0	4	4	0	0	0	0	0
17	100	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
18	100	0	0	0	0	0	0	0	0	2	0	1	1	1	1	0	2	0
19	100	0	7	1	2	10	2600	1	3	1	0	2	2	6	4	0	10	220
20	100	0	0	0	0	0	0	2	2	1	1	1	2	0	0	0	0	0
21	100	0	0	0	0	0	0	0	4	1	2	0	2	0	0	0	0	0
22	100	0	5	2	0	7	4980	1	0	1	0	1	1	8	4	0	12	1700
23	100	0	2	0	0	2	0	0	1	1	0	1	1	0	0	0	0	0
24	100	0	3	1	0	4	2360	-	-	-	0	0	0	0	0	0	0	0
25	100	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0	0
26	100	1	26	10	0	37	10520	0	0	2	0	3	3	16	5	0	21	1580
27	100	0	0	0	0	0	0	2	1	1	2	1	3	0	0	0	0	0
28	100	3	12	5	1	18	2860	2	0	1	0	1	1	1	2	0	3	40
29	100	0	1	0	1	2	180	0	1	1	1	0	1	3	2	0	5	560
30	100	0	10	0	0	10	22000	0	0	1	0	1	1	4	3	1	8	540

Group 2		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph.	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
31	100						260	18	11	1.5	4	0	4	1	1	0	2	660
32	100						5460	6	0	1	1	1	2	10	10	0	20	8040
34	100						5560	2	4	1	0	0	0	14	13	0	27	5240
35	100						0	3	1	1	1	0	1	3	0	0	3	20
36	100						0	0	1	1	2	0	2	0	0	0	0	0
37	100						20	2	1	1	0	0	0	0	0	0	0	0
38	100						0	0	0	1	1	2	3	0	2	0	2	0
39	100						8600	9	1	1.5	8	5	13	68	38	0	106	14560
40	100						0	0	0	1	0	0	0	3	1	0	3	200
41	100						0	0	0	1	0	2	2	0	2	0	2	20
42	100						0	1	0	1	1	0	1	0	2	0	2	0
43	100						0	4	5	1	4	7	11	2	7	0	9	1400
44	100						0	2	0	1	0	0	0	0	0	0	0	0
45	100						0	8	1	1	3	0	3	0	0	0	0	0

Group 2 cont.		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph.	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
46	100						20	0	1	1	0	0	0	7	3	0	10	1800
47	100						-	3	0	1	0	0	0	0	0	0	0	20
48	100						0	3	0	1	0	0	0	0	0	0	0	0
49	100						0	3	1	1	6	3	9	1	0	0	1	0
50	100						5700	3	2	1	0	8	8	7	4	0	11	7800
51	100						0	3	0	1	1	0	1	0	0	0	0	0
52	100						1660	1	2	1	2	1	3	2	1	0	3	1240
53	100						3700	0	1	1	2	6	8	6	7	0	13	1600
54	100						-	8	2	1	10	7	17	14	9	0	23	6080
55	100						0	1	2	1	0	0	0	3	1	0	4	360
56	100						0	0	4	1	1	0	1	0	0	0	0	0
57	100						20	3	1	1	0	2	2	2	3	0	5	0
58	100						0	1	0	1	0	2	2	3	2	0	5	0
59	100						2020	2	0	1	0	0	0	8	8	0	16	2800
60	100						0	1	0	1	0	2	2	7	3	0	10	800

Group 3		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
61	6000	0	0	0	0	0	-	3	0	1	0	0	0	0	0	0	0	0
62	6000	0	10	16	5	61	440	0	2	1	5	14	19	17	21	0	38	7560
63	6000	0	0	0	1	1	0	1	0	2	1	0	1	0	1	0	1	0
64	6000	0	0	0	0	0	0	3	3	1	14	5	19	0	0	0	0	0
65	6000	0	1	0	0	1	0	0	0	2	4	2	6	0	0	0	0	0
66	6000	0	0	0	0	0	0	0	1	1	2	0	2	0	0	0	0	0
67	6000	0	2	1	0	3	0	0	1	1	0	0	0	0	0	0	0	0
68	6000	0	1	1	1	3	1060	4	3	1	0	1	1	2	2	0	4	20
69	6000	0	1	0	0	1	0	6	5	2	28	6	34	7	3	0	10	1060
71	6000	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0
72	6000	0	0	0	0	0	0	-	-	-	1	3	4	0	1	0	1	0
73	6000	0	0	0	0	0	0	0	0	2	2	1	3	11	8	0	19	2100
74	6000	0	2	0	0	2	0	3	8	2	9	1	10	8	6	0	14	1000
76	6000	0	0	0	0	0	0	0	0	1	0	2	2	0	0	0	0	0

Group 3 cont.		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph.	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
77	6000	0	9	1	0	10	6560	0	1	1	0	0	0	0	0	0	0	3060
78	6000	0	0	0	0	0	0	5	1	1	0	0	0	0	1	0	1	0
79	6000	0	4	0	0	4	680	6	5	2	9	2	11	0	2	0	2	0
80	6000	0	5	2	0	7	500	0	0	1	1	3	4	2	0	0	2	0
81	6000	0	0	0	0	0	0	0	0	1	1	2	3	0	0	0	0	0
82	6000	0	0	1	0	1	0	0	2	1	6	6	12	5	6	0	11	14800
83	6000	0	0	0	0	0	0	1	0	2	1	1	2	2	0	0	2	0
84	6000	0	3	0	0	3	3600	0	0	1	0	0	0	0	0	0	0	0
85	6000	0	8	4	0	12	1060	0	1	1	0	0	0	0	0	0	0	0
86	6000	0	0	0	0	0	0	20	5	1	13	4	17	2	1	0	3	0
88	6000	0	0	0	0	0	0	0	0	1	1	3	4	6	2	0	8	0
89	6000	0	0	0	0	0	0	0	0	1	4	0	0	0	0	0	0	0
90	6000	0	1	0	0	1	0	0	1	1	1	0	0	0	0	0	0	0
92	6000	0	19	4	0	23	4800	0	0	1	2	3	5	4	1	0	5	0

Group 4		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph.	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
101	6000						720	0	0	2	0	0	0	4	0	0	4	0
102	6000						1540	0	0	1	0	0	0	4	1	0	5	1640
103	6000						9940	8	3	1	43	29	72	77	40	0	117	2760
104	6000						0	11	0	1	3	1	4	0	0	0	0	0
105	6000						0	0	0	1	0	0	0	0	0	0	0	0
106	6000						0	0	0	1	0	2	2	14	5	0	19	360
107	6000						0	0	0	2	0	0	0	0	0	0	0	0
108	6000						0	0	2	1	0	0	0	22	14	0	36	5400
109	6000						0	2	1	1	0	1	1	0	1	0	1	0
110	6000						0	171	27	2	41	0	41	5	1	0	6	560
111	6000						11920	2	0	2	1	2	3	23	6	0	29	16680
112	6000						0	0	0	1	0	0	0	5	0	0	5	0
113	6000						0	4	0	1	1	0	1	1	0	0	1	20
114	6000						0	36	0	2	5	0	5	0	0	0	0	0

Group 4 cont.		At Treatment						At Necropsy										
								Liver			Immature			Mature				
Pig	Dose	IMM	♀	♂	Un-ID	Total	EPG	WS diffuse	WS lymph.	LTS	<2mm	>2mm	Total	♀	♂	Un-ID	Total	EPG
115	6000						0	0	0	1	0	0	0	0	0	0	0	0
116	6000						9500	5	3	1	2	2	4	16	5	0	21	1800
117	6000						0	0	2	2	0	2	2	8	6	0	14	60
118	6000						-	137	18	1	96	6	102	116	73	0	189	29600
119	6000						0	0	2	1	1	2	3	2	1	0	3	640
120	6000						-	4	0	1	0	0	0	0	0	0	0	0
121	6000						2980	0	0	1	0	1	1	21	15	0	36	4640
122	6000						0	0	0	1	0	0	0	0	1	0	1	0
123	6000						0	0	0	1	0	0	0	2	0	0	2	0
124	6000						0	1	0	2	1	0	1	0	0	0	0	0
125	6000						0	13	1	1	11	0	11	2	2	0	4	1900
126	6000						0	0	0	1	2	0	2	2	1	0	3	700
129	6000						0	1	0	1	5	1	6	0	0	0	0	20
130	6000						0	0	1	1	0	0	0	6	2	0	8	40
131	6000						0	4	2	2	5	0	5	0	0	0	0	80

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